AD A088067

AFOSR-TR- 80-0596

ANNUAL TECHNICAL REPORT NO. 1

PROJECT A-2335

LEVEL

INVESTIGATION OF RADIOFREQUENCY RADIATION EFFECTS ON EXCITABLE TISSUES

By

E. C. Burdette and R. L. Seaman
Biomedical Research Branch
Electronics Technology Laboratory
Georgia Institute of Technology

and

R. L. DeHaan
Anatomy Department
Emory University School of Medicine

Prepared for

U. S. AIR FORCE
OFFICE OF SCIENTIFIC RESEARCH
LIFE SCIENCES DIRECTORATE
BOLLING AIR FORCE BASE, DC 20332

CONTRACT NO. F49620-79-C-0055

April 1980

GEORGIA INSTITUTE OF TECHNOLOGY

Engineering Experiment Station
Atlanta, Georgia 30332

1980

80 8 19 035

Unclassified

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER 19 AFOSR-TR-80-0596	2. GOVT ACCESSION NO. AD-A088	3. RECIPIENT'S CATALOG NUMBER 067 9
4. TITLE (and Subtitle) Investigation of Radiofrequency Radiation Effects on Excitable Tissues		5. TYPE OF REPORT & PERIOD COVERED Annual Technical Report 1 Mar 1979 - 29 Feb 1980
14 GIT/EES		6. PERFORMING ORG. REPORT NUMBER A-2335
7. AUTHOR(s) E. C. Burdette, R. L. Seaman, and R. L. DeHaan		8. CONTRACT OR GRANT NUMBER(s) F 49620-79-C-0055
9. PERFORMING ORGANIZATION NAME AND ADDRESS Engineering Experiment Station Georgia Institute of Technology Atlanta, Georgia 30332		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 61102F 2312A5 16 2312 17 A5
11. CONTROLLING OFFICE NAME AND ADDRESS Air Force Office of Scientific Research Director of Life Sciences/NL Building 410, Bolling AFB, D.C. 20332 (NL)		12. REPORT DATE 11 30 April 1980
		13. NUMBER OF PAGES 49 + v
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)		15. SECURITY CLASS. (of this report) Unclassified
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for Public Release, Distribution Unlimited

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

RF Exposure System
Coaxial Termination
Radiofrequency Radiation Bioeffects
Dosimetry
Absorbed Power
Excitable Tissues
Cardiac-Cell Aggregates
Interbeat Interval (beat rate)
Action Potential
Electromagnetic Fields

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The effects of 2450-MHz radiofrequency radiation (RFR) on excitable membranes were studied. Spherical aggregates of cultured chick cardiac cells were used as the model excitable tissue.

An exposure device was developed to deliver known amounts of RFR energy to the aggregate preparation. The device consists of a 1/4-inch semi-rigid coaxial cable which opens into a circular brass plate that serves as a ground plane. In our experiments, the brass plate is also used for regulated heating. next page

-continued-

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

Unclassified

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

165700

cont

The fringing field extending above the device aperture effectively couples to the culture medium in a 35-mm circular culture dish, with negligible radiated field outside the medium. The Specific Absorption Rate (SAR) inside the culture dish for two volumes of media was determined from measurements of electric fields and temperatures. Small dipole (2.9-mm) and monopole (2.7-mm) probes were used to perform field intensity measurements and 0.4-mm diameter high-resistivity thermistors attached to resistive leads were used to measure temperature rise. SAR was accurately determined as a function of input power and of position in the dish, with the maximum occurring at the center.

Electrical parameters of the cardiac-cell aggregates were measured before, during, and after RFR exposure. Action potential (AP) amplitude was not changed significantly (re pre-exposure values) for SARs of 8.4 to 480 mW/g of CW RFR and 1.7 to 112 mW/g pulsed (10.9 μs, 10 kHz) RFR. Changes in maximum upstroke velocity (MUV, in V/s) of the AP for the same SARs were small and variable from exposure to exposure, with some indication of greatest variability from 100 to 200 mW/g. Average interbeat interval (IBI, 60/beat rate) decreased for SAR >4 mW/g consistent with bulk heating effects. There was an indication that IBI variability increased with SAR, 0.3 to 300 mW/g. Changes in IBI were seen in microelectrode recording of APs and in video display of rhythmic contractions. The trends for greater variability have been suggested by all MUV and IBI data analyzed and are consistent with an RF perturbation of membrane molecular components. Additional studies are underway to see whether bulk heating can account for observed variabilities. (Sponsored by USAF Office of Scientific Research.)

- maximum upstroke velocity

- interbeat interval

ANNUAL TECHNICAL REPORT NO. 1

Project A-2335

INVESTIGATION OF RADIOFREQUENCY RADIATION EFFECTS ON EXCITABLE TISSUES

By

E. C. Burdette, R. L. Seaman, and R. L. DeHaan*

APRIL 1980

Prepared for

U.S. Air Force
Office of Scientific Research
Life Sciences Directorate
Bolling Air Force Base, DC 20332

Prepared by

Biomedical Research Branch
Electronics Technology Laboratory
Engineering Experiment Station
Georgia Institute of Technology
Atlanta, Georgia 30332

* Anatomy Department, Emory University School of Medicine, Atlanta, GA 30322

FOREWORD

Research during the first year of this two-year program was carried out by personnel of the Biomedical Research Branch of the Electronics Technology Laboratory of the Engineering Experiment Station at the Georgia Institute of Technology, Atlanta, Georgia 30332 and by personnel from the Anatomy Department of the Emory University School of Medicine, Atlanta, Georgia 30322. Mr. E. C. Burdette served as the Principal Investigator. The research program, which is sponsored by the U.S. Air Force Office of Scientific Research, Bolling Air Force Base, D.C. 20332 under Contract No. F49620-79-C-0055, is designated by Georgia Tech as Project A-2335. This Annual Technical Report summarizes the work which was performed during the period from 1 March 1979 through 29 February 1980.

This work was made possible through the combined efforts of many people at the Air Force Office of Scientific Research (AFOSR), at the U.S. Air Force School of Aerospace Medicine (USAFSAM, Brooks Air Force Base, Texas), at the Emory University School of Medicine, and at the Georgia Institute of Technology. The authors would especially like to thank Dr. W. O. Berry at AFOSR, Mr. J. C. Mitchell, Dr. J. D. Krupp, and Mr. J. H. Merritt at USAFSAM, and Mr. J. C. Toler and Mr. F. L. Cain at Georgia Tech, all of whom contributed significantly to the first year's success of this research program.

Respectfully submitted,

Everette C. Burdette

Everette C. Burdette
Principal Investigator

Approved:

J. C. Toler

J. C. Toler, Head
Biomedical Research Branch

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF FIGURES

-Continued-

LIST OF FIGURES

-Concluded-

LIST OF TABLES

SECTION I
INTRODUCTION

A. Research Objectives

The overall objective of this program is to investigate the effects of pulses- and continuous-wave radiofrequency radiation (RFR) on the electrical properties of cardiac-cell aggregates, both singularly and when joined together as chains. Specific research objectives include the investigation of

1. RF exposure system designs which offer well-characterized dosimetry and are suitable for cellular-level RF exposure,
2. alterations in spontaneous rhythm and other action potential characteristics (resting potential, threshold potential, maximum upstorke velocity) during RFR exposure,
3. alterations in specific slow- and fast-channel ion currents during RFR exposure, and,
4. modification of pharmacological agent interaction with ionic channels.

B. Review of Radiofrequency Fffects on Excitable Tissues

Interest in the effects of radiofrequency radiation on biological systems has existed for quite some time. In many cases, interest has focused on the effects of RFR on excitable tissues. The normal function of these tissues involves changing electric fields and ionic currents which can be altered by applied static and slowly changing electromagnetic fields. It is conceivable that higher-frequency fields, such as RFR, could also interact with the naturally occurring fields of these tissues. In this section, a brief review of the literature on cardiac effects and other topics relevent to the subject of this report are presented.

Some of the earliest research of RFR cardiovascular effects is reported in the Soviet literature. Presman summarizes these and as well as effects found at other frequencies in his research monograph [1, pp. 116-124]. A series of experiments were performed using rabbits exposed to pulsed- and continuous-wave (CW), 2400- or 3000-MHz radiation at incident power densities of

3-5 or 7-12 mW/cm^2. Small increases and decreases in heart rate were observed depending on the body region exposed. It was concluded that pulsed radiation had a stronger effect than did CW radiation. Decreases in heart rate were attributed to stimulation of peripheral nervous systems since they were absent when the exposed skin was anesthetized. Increases in heart rate were attributed to effects on the central nervous system and were dominant when only the head was exposed.

Replications of these studies have been carried out using 2400-MHz CW and pulsed radiation [2,3]. With CW energy applied only to the head, no significant changes in heart rate were observed with 10 mW/cm^2 incident power density and only the highest level used, 100 mW/cm^2, was effective in increasing heart rate. With CW or pulsed RF energy applied at 20 mW/cm^2 to the entire dorsal surface of the rabbit, no difference in response was seen. Respiration rate was found to be more sensitive than heart rate in these studies. Previously reported changes in heart rate were attributed to chance variations.

Various criticisms have been directed at the above Soviet studies (4, p.121]. Major among these are the use of exposure conditions with different field characteristics (far-field exposure in one case and near-field in the other) in the different studies and the necessity of using enough animals to detect small changes. We also add to these criticisms by noting the differences in power levels. Thus, it can be seen from the various studies that the effects of low level RFR on heart rate in an intact animal are far from resolved.

An additional criticism of a number of studies of potential RFR effects on excitable tissues is the lack of adequate useful dosimetry information, including such problems as near-field versus far-field distributions and how these exposure differences could affect the observed biological parameters. Therefore, a significant objective of our work has been the development of a well-characterized exposure device.

Isolated heart preparations have been used in attempts to delineate direct effects on cardiac tissue. In denervated frog hearts, no change in heart rate was seen in response to an incident field of 0.06 mW/cm^2, while exposure of intact frogs produced effects similar to those which had been observed in rabbits [1, p. 122]. Pulsed radiation at 1425 MHz at low repetition rates was found to be effective in causing arrhythmias

in isolated frog hearts but only for certain delays from the occurrence of the P wave of the electrocardiogram [5]. Pulse duration was 10 microseconds (μs) and pulse rate was synchronized with the P wave such that pulses were radiated conincident with the P wave, 100 milliseconds (ms) after the P wave and 200 ms after the P wave. In 50 percent of the irradiated cases, arrhythmias associated with the radiation were observed. However, similar studies have failed to confirm this effect [6,7].

Some interesting results have been obtained from isolated hearts using CW radiation at 960 MHz applied with a capacitor irradiator [8-11]. For turtle hearts, Specific Absorption Rate (SAR) in the range of 2 to 10 mW/g at 960 MHz caused a decrease in heart rate while large SARs and generalized heating caused an increase in heart rate [8,9]. Results from drug studies suggest that the decreases seen at small SARs are due to neurotransmitter release from nerve remnants in the heart [9]. Similar decreases in heart rate have been found in isolated rat hearts at absorbed powers of 1.3 to 2.1 mW/g [10,11] and drug studies again indicate an interaction with nerve remnants [11]. These findings from isolated hearts indicate possible interaction of RFR with some nerve components by means other than purely thermal mediation.

Pacemaker neurons from an invertebrate have also been studied [12,13]. These neurons were exposed to 1500- and 2450-MHz radiation in a stripline exposure device and their transmembrane potentials were recorded during exposure. Both decreases and increases in firing rate were observed, with some changes occurring for SARs as low as 2 mW/g. Most slow changes in rate were reproduced by warming. However, those cases which were not reproduced and the finding of changes occurring too rapidly to be caused by gross heating indicated that processes other than thermal were operative.

There have been attempts to explain possible mechanisms for RFR interaction with excitable tissues. Directly-induced transmembrane potentials have been estimated to be on the order of a few hundred microvolts in the central nervous system of animals exposed to an incident field power density of 10 mW/cm^2 [14]. Rectification of induced microwave currents to produce effective direct currents has been proposed [12] and can be predicted on

the basis of nonlinear conductance [15] or capacitance [16] in the membrane. However from a microscopic-level analysis, it has been concluded that rectification cannot be effective at frequencies above an estimated 32 MHz because of transit time effects, and that effects on individual membrane particles are probably more significant than rectification at higher frequencies [17]. This is plausible since not all of the effects seen in neural pacemakers can be explained in terms of equivalent direct currents [13].

From this brief view, it can be seen that the effects of RFR on excitable tissues are controversial, with conflicting results often reported. In addition, the mechanisms by which RFR can interact with these tissues are presently not well defined. It is against this background that the cardiac-cell aggregate system is being used to define effects on RFR more completely and to investigate various mechanisms.

C. Brief Summary of Results and Report Organization

During the first year of performance on this research program, there have been several significant goals achieved. An effective exposure device, described in Section III of this report, has been developed and characterized for use at 2450 MHz. The cardiac-cell aggregate preparation, described in Section II, has proven to be a useful system for probing effects on RFR on excitable membranes. Spontaneous rhythm and action potential maximum upstroke velocity have been found to be more sensitive to RFR than other aggregate properties and will be used for further work. Decreases in beat rate with RFR were consistent with changes due to bulk heating. Increased variations in beat rate and maximum upstroke velocity were seen under RFR exposure conditions and could be due to RFR-induced alterations in the opening and closing of ionic conductance channels. These results are described in Section IV and conclusions and recommendations based on these results are presented in Section V.

SECTION II
CARDIAC-CELL AGGREGATES

A. Preparation and Electrical Characteristics

For the investigations described in this report, measurements have been made on spheroidal aggregates of embryonic chick heart cells maintained in tissue culture. After incubation for 7 days at 37.5^{o}C, white Leghorn chicken embryos were harvested in amniotic fluid and decapitated. Their hearts were dissected free, trimmed of extraneous tissue, and the ventricles were dissociated with trypsin by techniques which have been previously reported [18-20]. Aggregates were prepared by placing an inoculum of 5×10^5 cells in 3 ml of culture medium (818A) on a gyratory shaker. Cells were allowed to aggregate during 48 hours gyration (62 rpm, 37.5^{o}C) in an atmosphere containing 5% CO_2, 10% oxygen, and 85% nitrogen. At the end of the gyration period, each aggregation flask contained up to 200 spheroidal clusters of heart cells, which ranged in diameter from 60 to 250 μm and contained 10^2 - 10^4 cells each. These aggregates are composed of several hundred individual cells beating spontaneously and rhythmically in a coordinated fashion. A field of newly formed aggregates is shown in Figure 1. These aggregates can be joined experimentally to form groups or chains as shown in Figure 2. When held together for a few minutes, aggregates adhere and form low-resistance electrical coupling junctions at their apposed surfaces, which allow all joined aggregates to take on a synchronous beat [21,22]. Individual beating aggregates develop a static electric field (associated with the action potential) that builds and collapses with each beat. Aggregate chains, in contrast, set up longitudinal paths of ionic current through the chain and surrounding medium that last as long as the action potential (200-300 ms) associated with each beat.

For impalement with microelectrodes, aggregates were allowed to adhere to the surface of a Falcon® plastic tissue culture dish maintained on a microscope warm stage under conditions in which pH, temperature, gaseous atmosphere and evaporation could be controlled and mechanical vibration minimized. In these circumstances, aggregates continued beating rhythmically for many hours. In most of our experiments, a microelectrode pulled from

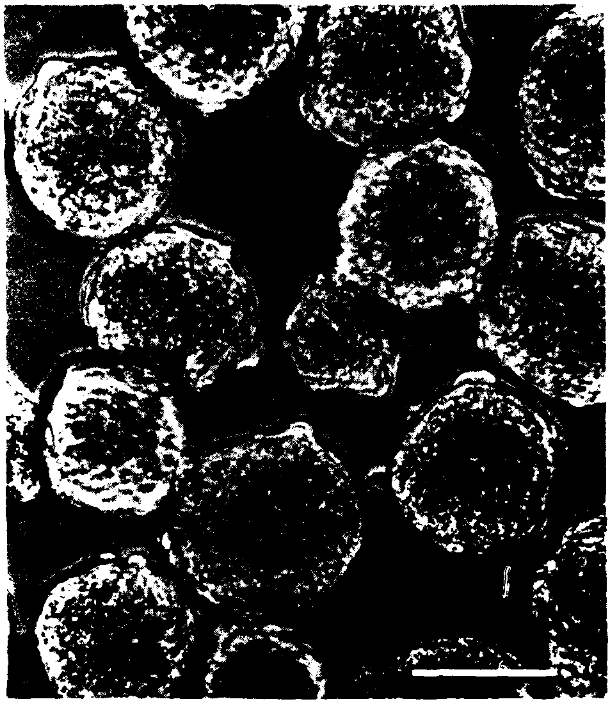


Figure 1. Field of spheroidal heart cell aggregates after 48 hrs of gyration culture. Scale - 100 μm.

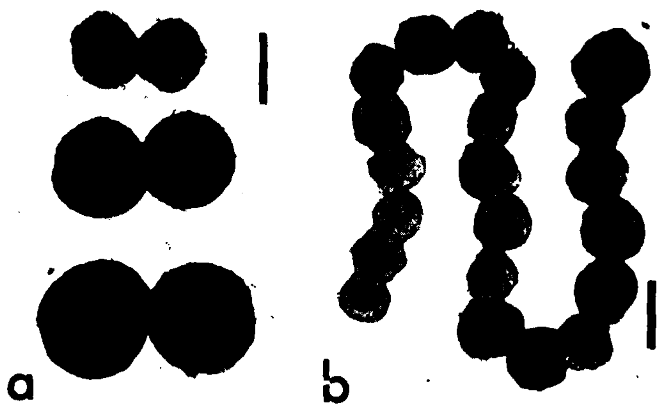


Figure 2. Linear configurations of spheroidal aggregates.
a. Aggregates arranged in equal-sized pairs, photographed 20 minutes after initial adhesion. Only the smallest pair had achieved beat synchronization; after an additional 20 minutes, all do. Scale: 100 μm.
b. A chain of 21 aggregates, photographed about 90 minutes after construction; all of the aggregates in the chain were beating in synchrony. Scale: 200 μm.

capillary glass tubing and filled with 3M KCl was used to penetrate an aggregate to record electrical activity. Transmembrane potential was amplified by preamplifiers having a very high input impedance and the amplified signal was displayed on a storage oscilloscope and recorded on magnetic tape for off-line analysis. In the reported studies, the action potential parameters analyzed included maximum diastolic potential (MDP), maximum positive potential (MPP), and maximum upstroke velocity (MUV). MDP is the maximum total potential difference between resting potential and zero potential, and MPP is the positive extent of the action potential. MUV is the largest rate of voltage change during the rapid change to maximum positive potential. In addition, interbeat interval (IBI), the time between action potentials (which is the reciprocal of beat rate converted to seconds) was measured.

To record spontaneous beats of aggregates or aggregate chains undisturbed by electrode penetration or other invasive observational techniques, beating activity was monitored in some experiments with the aid of a closed-circuit video system, shown in Figure 3, which had been modified from that described previously [23,24]. A closed circuit television (TV) camera (Panasonic WV-400P) positioned at the photo-ocular of a microscope, projected the image of an aggregate onto a TV screen. Beats were recorded by placing a phototransistor sensor on the video screen over the edge of the image of an aggregate. Signals from the phototransducer were displayed on an oscilloscope (Tektronix R5103/D13) and stored on magnetic tape for off-line analysis. The recorded signals were processed with a PDP 11/40 computer (Digital Equipment Corp) with the aid of a Schmitt trigger. Before analysis, records were amplified and filtered with a Kron-Hite 332 narrow band-pass filter (cut-off frequency $0.1<f<20-30$ Hz). Standard computer algorithms were used to compute interval histograms with a 5-ms bin width. Statistical analyses are described in Section IV.

The multicellular nature of heart tissue normally places severe limitations on the ability to investigate its electrical and molecular properties. The fact that cardiac muscle is composed of individual cells connected by junctions

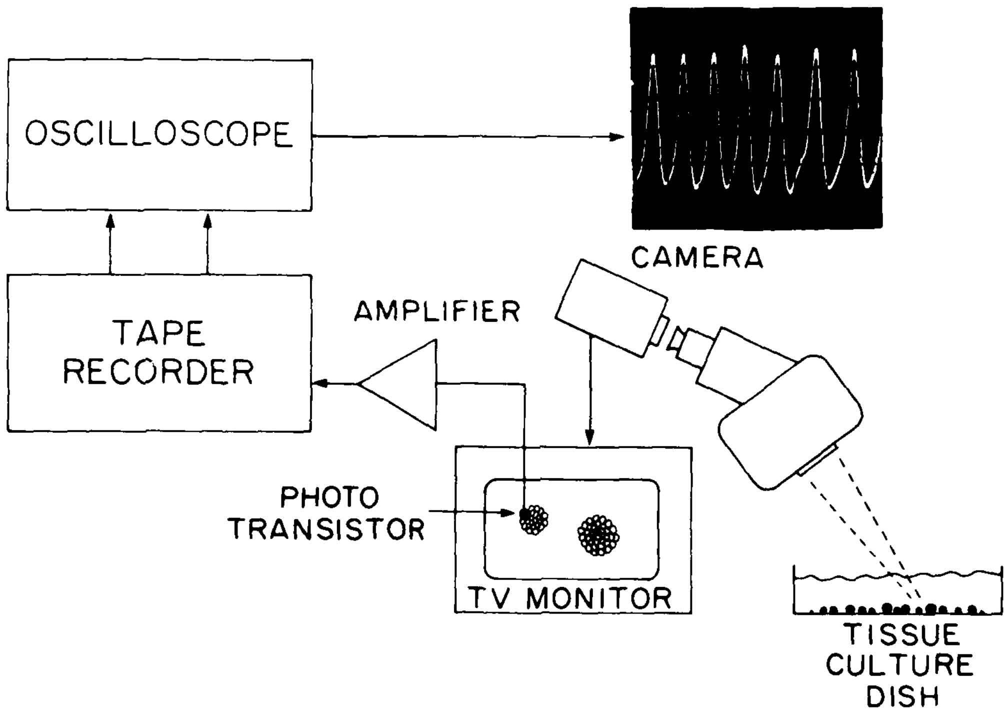


Figure 3. Block diagram of the video recording apparatus used to obtain trains of interbeat intervals. Insert: a typical record of the moving edge of an aggregate, showing the output of the photo-transistor applied to the image on the monitor screen.

of low but varying resistance into fibers of complex geometry [25,26] guarantees that voltage gradients must exist within such fibers, and currents crossing the membrane of one cell may differ from those flowing in a distant cell. In 1972, the spheroidal heart cell aggregate was introduced as a tissue culture model for studies of the electrical properties of the heart cell membrane [18,27]. We have demonstrated that such aggregates offer numerous advantages over more traditional cardiac preparations.

1. All cells within an aggregate are closely coupled by low-resistance junctions (Figure 4) while the non-junctional myocyte membrane exhibits large resistive, capacitive and inductive elements [28]. For spheroids of 200 μm diameter or smaller, signals in the frequency range 0-20 Hz are experienced essentially simultaneously throughout the entire membrane [19,29]. Thus, each aggregate approximates an isopotential system for both spontaneous and imposed signals as indicated by Figure 4.

2. Because of these properties, the aggregate action potential approximates a true non-propagated membrane potential, for which the relation

$$dV/dt = I/C \qquad (1)$$

is applicable and the maximal slope of the action potential upstroke (MUV) is a good measure of total inward current, which is the sum of the sodium and slow inward currents.

3. Aggregates can be prepared in any size within a 30-300 μm diameter range from a few cells to several thousand cells, containing 10^{-7} to 10^{-2} cm^2 of membrane. Because of their nearly spherical shape and the known amount of intercellular space, the total cell surface contained within an aggregate can readily be calculated from the aggregate diameter [20,30], taken as $2(ab)^{1/2}$, where a and b are the major and minor hemiaxes seen from a top view of the preparation. Total aggregate volume (V_a) is obtained from $V_a = 4/3\ \pi ab^2$. Since 20% of V_a equals extracellular space, the number of

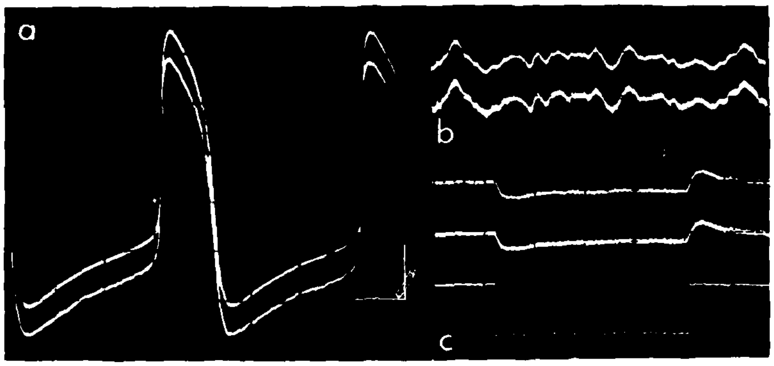


Figure 4. Demonstrations of voltage homogeneity in heart cell aggregates. a. Spontaneous action potentials recorded simultaneously through microelectrodes in two cells 223 μm apart at opposite poles of a 260 μm diameter aggregate. The two traces are displaced vertically in order to distinguish them. At a sweep speed of 0.5 msec/div the upstrokes of the two action potentials can be seen to be separated by approximately 50μ sec. Scale: 20 mV, 100 msec.

b. Voltage noise recorded from two cells 118 μm apart in a single 154 μm diameter aggregate. Spontaneous action potentials have been suppressed with 3×10^{-6} M tetrodotoxin. Potential fluctuates aroung a mean resting potential of -51 mV, producing typical "voltage noise". Scale: 1 mV, 1 sec.

c. Voltage responses (upper and middle trace) to a 2 nA hyperpolarizing current pulse (lower trace) recorded from two cells 115 μm apart in a 193 μm diameter aggregate, made quiescent with tetrodotoxin. Resting potential -51 mV. Scale: 20 mV, 2 nA, 1 sec.

Figure 5. Phototransistor output recording of 15 consecutive beat intervals from two different cell clusters in the same culture dish under identical conditions. (a) 2-cell group, mean IBI, 0.465 sec; variation around mean = 23.5%. (b) 75-cell group, mean IBI, 0.49 sec; variation around mean = 3.9%. Scale = 200 msec.

cells per aggregate (N) can be calculated from 0.8 V_a/V_c where the mean cell volume (V_c = 600 μm^3) was derived from a mean cell diameter d of 10.8 μm (Atherton and DeHaan, unpublished), and the total cell surface is calculated as $N\pi d^2$. We have also estimated N by measuring aggregate DNA content, using an observed value for DNA per cell of 2.8 picogram measured in single ventricle cell suspensions and in chicken erythrocytes. Total DNA per aggregate was determined by the method of Giles and Myers from pooled groups of counted and sized aggregates. The mean cell volume calculated from these DNA assays was 651 μm^3, and the values of N determined from the ratio total DNA/cell DNA agreed within $\pm$ 1% with those calculated from measurements of aggregate diameter. The total membrane area of an aggregate 150 μm in diameter is $7.8 \times 10^{-3} cm^2$.

4. Aggregates are "sticky"; they can be formed into pairs, chains, or clusters (Figure 2) in which electrical coupling junctions are quickly established at points of contact. Such multi-aggregate systems soon take on a coordinated, synchronized beat. Diffusion of ions and other molecules into and out of the intercellular tissue spaces is rapid.

5. Aggregates can be prepared from hearts from any desired embryonic age, from stages prior to the time the organ begins to beat to hatching or shortly thereafter. Such spheroids reflect accurately the physiological and pharmacological properties of the intact donor tissue.

6. Aggregates can be maintained in healthy condition for many days in culture, while maintaining spontaneous and rhythmic beating.

7. Because they are naturally "space clamped", that is, virtually isopotential over a reasonable range of frequencies, aggregates can be subjected to voltage clamp analysis of their current-voltage relationship [20]. This capability has recently been utilized to show directly the appearance of sodium channel current I_{Na} in aggregates from 3-day hearts maintained for an additional 3-5 days in culture [31].

B. Spontaneous Beat Fluctuations

The rhythmic beat of the heart is its most striking characteristic, but the rhythm is not as regular as it seems. Consecutive interbeat intervals (IBIs) in the spontaneous heartbeats of healthy adults are not identical; rather there is a small, apparently random variation of about 2% around an ideal mean interval. Heart cell aggregates beating spontaneously in culture also exhibit a fluctuation of about 2% around a mean IBI, but for small cell clusters (smaller than 100 cells) the spontaneous fluctuation in IBI increases inversely with N, the number of cells comprising a cluster [24]. For example, a cluster comprised of 75 coupled cells showed a spontaneous fluctuation in IBI of 3.9%, whereas consecutive IBIs in a 2-cell group varied by 23.5% from the mean as shown in Figure 5. It has been recently argued [24] that the spontaneous fluctuation in IBI should be directly related to the amplitude of membrane voltage noise (Figure 4b) and that a perturbation that alters voltage noise should also affect the fluctuation in IBI. We have recently shown that a major component of membrane voltage noise stems from the random opening and closing of voltage-sensitive specific ion channels [28] so that a perturbation of ion channels should cause a change in fluctuation.

C. Temperature Effects

The spontaneous beat rate of heart cell aggregates -- like all biological processes -- is sensitive to temperature changes. In the range 15-39°C, heart tissue speeds up its beating (i.e. IBI becomes smaller) when it is warmed. As shown in Figure 6, aggregates are more sensitive to temperature at the low end of that range than at higher temperatures. For example, a 1°C increase in bath temperature from 20 to 21°C causes aggregate IBI to shorten from 8.6 sec to 6.9 sec, a decrease of 20%. A similar 1°C increase in bath temperature from 36.5 to 37.5°C, however, causes only an 11% decrease in the IBI from 1.4 to 1.25 sec. In more familiar terms, when an aggregate at 37°C, beating at a rate of 50 beats per min, is exposed to an increase in environmental temperature of 1°C, its rate increases to about 56 beats per minute.

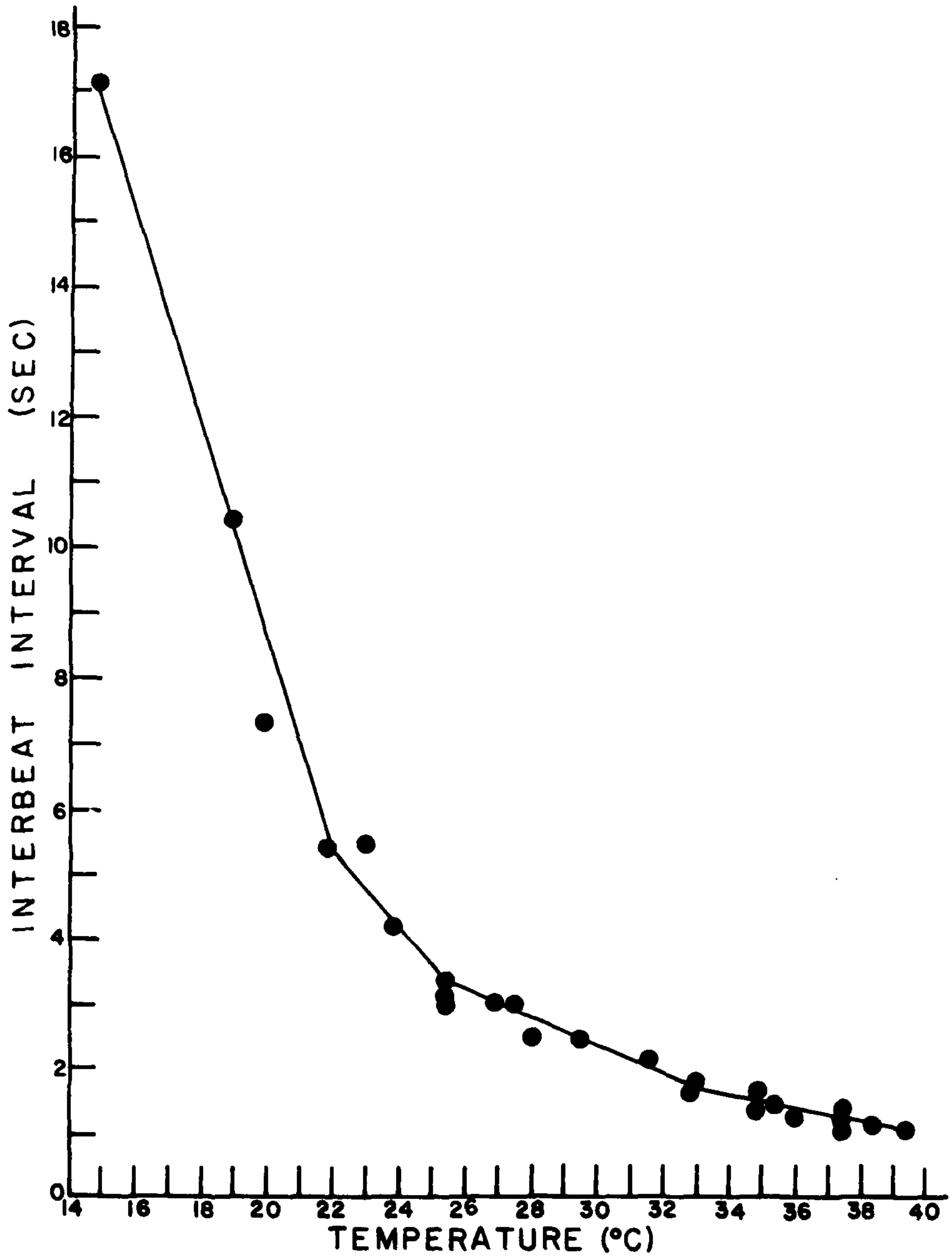


Figure 6. Effect of ambient temperature on mean IBI of 150-μm ventricular aggregates prepared from 7-day heart culture.

SECTION III

RADIOFREQUENCY RADIATION EXPOSURE SYSTEM DEVELOPMENT

A consideration in every study of the possible effects induced in biological systems by exposure to RF fields is that of dosimetry. Since the beginning of investigations involving the interaction of RF radiation and living organisms, the need to know accurately the EM power absorbed by the exposed organism has been an important and necessary factor in determining exposure levels and/or absorbed dose relative to personnel safety. In previously reported studies of RFR effects on excitable tissues, very few of the reported experiments give dosimetry information adequate to permit an accurate assessment of the exposure conditions under which the observed results occur.

A. Review of Design Approaches

Three candidate systems for the RF exposure of cardiac-cell aggregates were initially considered and evaluated. Those exposure configurations were (1) a horn illuminator for free-space exposure conditions, (2) an in-line exposure configuration in which the aggregates are placed within an enclosed waveguide, and (3) a waveguide slot radiator in which the culture dish containing aggregates is placed over the slot. To those exposure system design concepts, two additional aggregate exposure configurations were added: a coaxial slot radiator and an open-ended termination coaxial exposure device. The first two of the original exposure schemes were rejected because of stray radiation and reflections in the case of the horn and because of anticipated difficulties with optical illumination of the preparation in the case of the waveguide. In addition, access to the interior of the waveguide would require special consideration with regard to waveguide fabrication. By placing the preparation over a waveguide or coaxial slot, the microwave energy would be applied from the bottom and microelectrodes would approach from the top using conventional techniques. It was expected that this physical separation of fields and electrodes would result in an electrical isolation of the electrodes from the microwave fields. With this configuration, there

would certainly be no problem with access to the exposure site. Optical illumination would necessarily be from the side as has been done previously with this preparation. Both of the slot exposure systems and the terminated coaxial exposure system were evaluated experimentally to determine the approach best-suited for the exposure of cardiac-cell aggregates to RFR.

B. Experimental Evaluations of Candidate Exposure Systems

In order to deliver known quantities of microwave energy to cardiac cell aggregates to be studied in this project, development of a suitable exposure device was undertaken. The device should efficiently provide a controlled amount of energy at the aggregate in a 35 mm circular culture dish containing a few milliliters of physiological medium. It was also desired that the interaction between the microwave fields and the microelectrodes used for recording electrical parameters be as small as possible.

The waveguide slot method of exposure was the first method to be tried. A slot was cut into the broad wall of S-band waveguide with the slot's long dimension transverse to the waveguide axis. The center of the slot was placed on the center line of the broad wall. The culture dish with 3 or 4 ml of solution was centered over the slot and rested upon the waveguide. Four slot sizes were evaluated. These ranged from 1.5 mm by 7.0 mm to 6.0 mm by 22.5 mm. The two smaller slots did not radiate. The two larger slots did radiate and the smaller of these (2.0 mm by 22.0 mm) was designated as the "narrow slot", while the larger slot (6.0 mm by 22.5) was designated as the "wide slot."

Measurements of the initial temperature rise resulting from a step input of microwave power were made and used to calculate a Specific Absorption Rate (SAR). The temperature measurement was made at the center of the dish using a high-resistivity thermistor with resistive leads ($\approx 80 k\Omega/m$) placed orthogonal to the electric field. An example of the measured temperature rise, showing initial linear transient region and steady state, is presented in Figure 7. The SAR was normalized by dividing it by the input power to the device being studied. This normalized SAR was subsequently used to compare the deposition of microwave energy in the physiological medium by the various exposure devices.

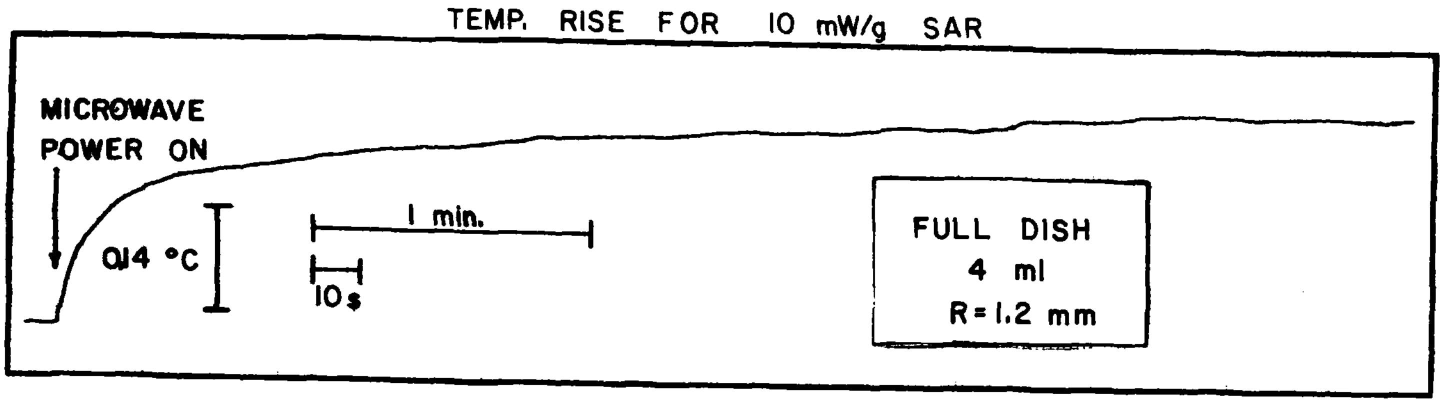


Figure 7. Typical recorded results for temperature rise measured using high-resistivity thermistor with resistive leads. SAR was computed from the initial rate of temperature change in the bathing medium at the onset of microwave power. Case shown is for an SAR of 10 mW/g.

The SAR was calculated for the culture dish containing 4 ml of balanced salt solution also for smaller volumes of this solution contained in wells at the center of the dish. Each well was a hole which was fashioned by removing a central portion of paraffin which filled the dish to a depth of 6 to 8 mm. The solution geometries employed in the evaluation of the waveguide slots were the following:

- full, or open, dish,
- large circular hole, approximately 15 mm diameter.
- small circular hole, approximately 10 mm diameter, and
- slot, approximately 10 by 22 mm.

Measurements were made on the slot with its long dimension either orthogonal to or parallel to the waveguide axis, termed "transverse" and longitudinal", respectively.

For every configuration, the wide slot delivered more energy to the medium than did the narrow slot. This agrees with the observation that the wide slot affected power transmitted through the waveguide more than did the narrow slot, indicating more radiation from the wide slot. The greatest normalized SAR measured, 12.2 mW/g/W, was for the full dish over the wide slot.

Measurements of SAR were also made using a slot in an RG-8/U coaxial cable used as the exposure device. The coaxial slot consisted of a hole in the braided outer conductor of the cable. The hole was roughly rectangular in shape having dimensions of 8 and 10 mm, with the longer dimension parallel to the cable length. The culture dish rested lightly upon the sides of the hole and the exposed dielectric. Additional stability for the dish was provided by dielectric supports at the sides of the cable. As shown in Figure 8, the normalized SARs for the coaxial slot were similar to those found for the waveguide slots, except in the case of the small circular hole dish geometry. It was concluded that there was not a great enough difference between the coax and waveguide methods to recommend one over the other on the basis of efficiency.

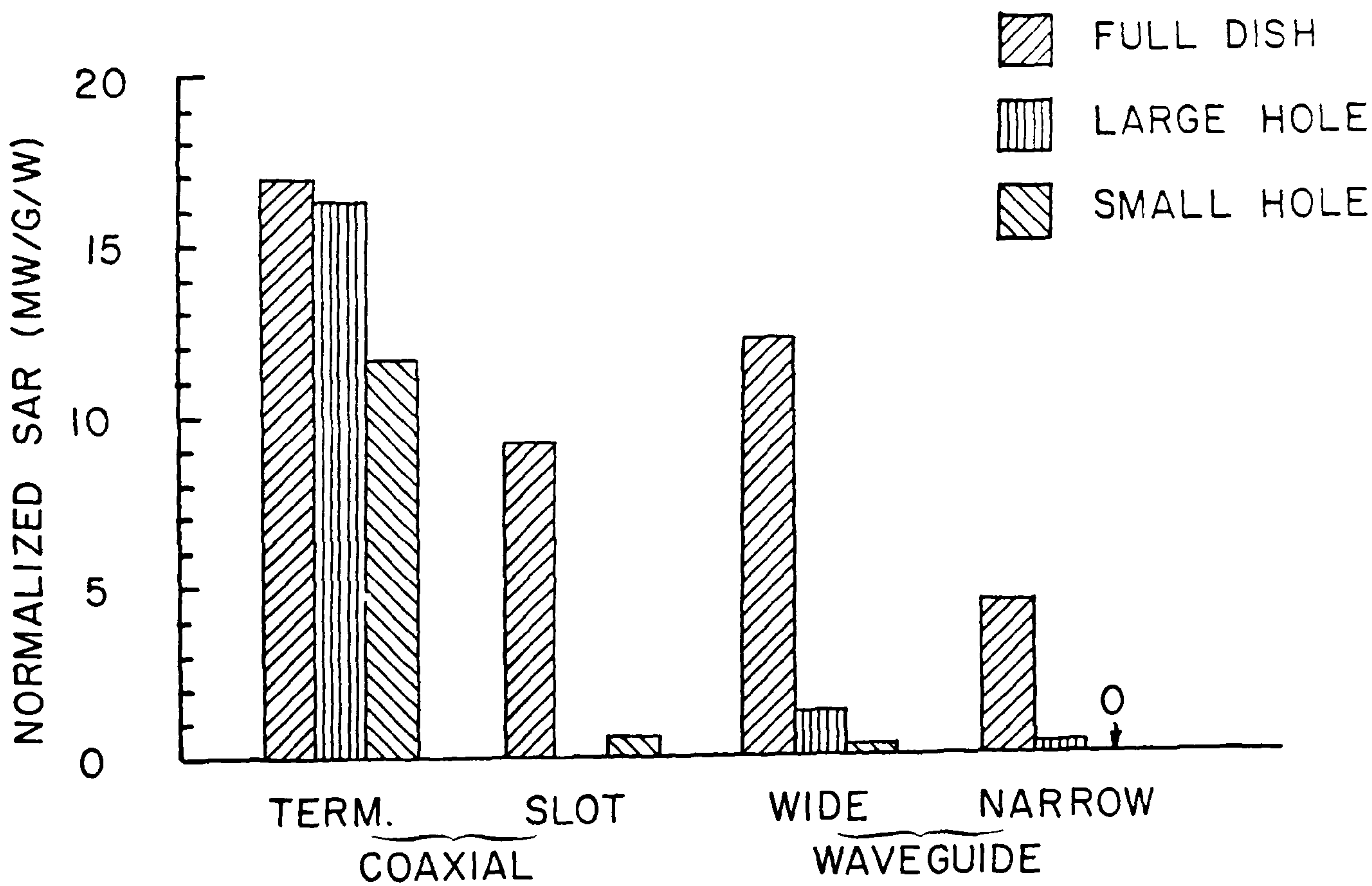


Figure 8. Specific absorption rate in culture dishes over different exposure devices and normalized to the input power of the devices.

Summarizing the performance of the slot radiators, it was found that more microwave energy was deposited using the larger radiating slots and for greater volumes of aggregate medium. The power levels measured using two immersed probe antennas (horizontal dipole and vertical monopole; described in Section III.D) agreed well with the computed SAR results. The radiation fields which were necessary to couple energy to the medium extended significantly beyond the boundaries of the culture dish. This radiated power was unacceptable for two reasons. First, the field within the medium was affected by the movement of nearby objects located within the radiated field. Objects separated from, but in the vicinity of the dish, influenced the measured field configuration within the dish. This effect was most readily observed when a metal plate was moved in the vicinity of the culture dish over either the coaxial or waveguide slot. In some cases, the field measured inside the dish varied as much as a factor of ten. Secondly, the extended field occupied the region through which recording electrodes would pass.

In considering the problem with exposing the cardiac aggregates in a radiation field, particularly in view of the need for impaling the aggregates with KCl-filled glass microelectrodes for recording purposes, it was decided to investigate a technique which would couple the microwave energy to the aggregate-containing medium in a capacitive field. Such a technique should reduce or eliminate the field perturbation effects encountered with the radiation field devices. The technique involved the use of an open-ended coaxial cable terminated by the culture dish. In a prototype device, a ground plane was attached to the end of a cable which was held in a vertical position by a clamp near the ground plane.

Measurements of the temperature rise with a rapid onset of microwave power were performed and used to compute SAR at various locations along the radial dimension of the culture dish, taking advantage of the circular symmetry of both the dish and the end of the coax. At the center of the dish, the normalized SAR values computed were large; however, this may in part be due to a possible artifact induced by the parallel alignment of the electric field and the thermistor leads at that location. At locations approximately midway between the center conductor and the outside of the ground plane hole, the computed SAR values were very reliable because the

electric field was orthogonal to the leads of the thermistor used to measure temperature. At distances greater than a centimeter outside the circumference of the ground plane hole, the SAR was effectively zero.

Figure 8 is a comparison of the normalized SARs for the various exposure devices in regions with horizontal electric fields (orthogonal to thermistor probe). These particular values are chosen on the basis of the minimal direct coupling of the fields with the thermistor used to measure temperature and the utility of the horizontal field in the experimental protocols with cardiac aggregates. For the full dish and the circular wells, the coaxial cable terminated with the culture dish is more efficient (larger normalized SAR) than the other exposure devices. As a result, the terminated coaxial cable was chosen for development because of the following characteristics:

- very small amount of radiated energy resulting in accurate dosimetry and minimal interaction with nearby objects,
- circular symmetry to match the culture dish geometry,
- horizontal radial electric field about which chains of aggregates can be oriented and which were expected to couple minimally to the vertically-oriented electrodes to be used, and
- ready incorporation into the existing recording setup.

The performance of the final terminated coaxial exposure device is described in detail in Section III.D of this report.

C. Dielectric Measurements of Cardiac-Cell Bathing Media

The physical interaction of an electromagnetic wave with the cardiac-cell aggregates in their bathing medium is primarily dependent upon the dielectric properties of the aggregates and the bathing medium. Further, a knowledge of these dielectric properties is necessary if the deposited RF power and field distribution within the culture dish are to be accurately determined using analytical methods. Dielectric measurements of the media used with the cardiac cell aggregates were performed using a probe technique developed at Georgia Tech [32,33]. The dielectric properties of the aggregate culture medium and a balanced salt solution were measured at a

frequency of 2450 MHz for temperatures between 24°C and 40°C. Dielectric properties of the two media were similar over this temperature range, with the exception being a higher dielectric constant for the culture medium at higher temperatures. Measurement results for the balanced salt solution and culture medium are presented in Tables I and II.

D. Dosimetry and Performance Characterization of Coaxial Exposure Device

Significantly improved performance over the slot radiators was obtained by developing an exposure device consisting of a coaxial cable opening into a ground plane. In tests of open-ended coaxial exposure devices, it was found that no measurable radiated field existed beyond the culture dish. In addition, there was more energy coupled to the medium for the same power into the device than for any of the radiating slots. Figure 9 is an isometric projection of the open-ended coaxial device which has been fabricated for use in the RFR exposures of cardiac-cell aggregates. The device consists of a 1/4-inch semi-rigid coaxial cable which opens into a circular brass plate that serves as a ground plane. The brass plate is also the heating plate which is used to maintain the desired temperature of the culture dish placed on it.

The RF fields produced by this device at 2450 MHz within the medium in a culture dish have been characterized by measurements of electric fields and temperature changes. The recording setup used in our laboratory for characterizing the exposure device is diagrammed in Figure 10. Two small probe antennas were used for the field measurements. One probe was a vertical monopole and the other a horizontal dipole. Initially, the field probe measurements were manually recorded on a point-by-point basis. Because this recording method proved to be tedious, time-consuming, and of questionable accuracy, a system for recording power received by the antennas continuously as a function of position was developed. Plots of received power for the antennas were recorded for vertical and horizontal sweeps through the dish. The maximum horizontal field component was located approximately midway between the vertical projections of the coaxial center and outer conductors and the maximum vertical field occurred at the center of the dish. The power levels received by the small field probes as a function of position within the medium are presented in Figures 11 and 12. The effects of medium volume were also studied and it was found that the measured field intensity was somewhat dependent upon the depth of the medium.

TABLE I

DIELECTRIC PROPERTIES OF AGGREGATE CULTURE MEDIUM 818A

Temperature (°C)	Dielectric Constant	Conductivity (mmho/cm)	Loss Tangent
24	75.3	30.43	.296
26	74.7	30.27	.297
28	73.9	30.06	.298
30	72.4	29.69	.301
32	70.5	29.22	.304
34	67.7	27.99	.303
36	64.6	27.29	.310
38	61.3	26.04	.312
40	58.8	25.01	.312

TABLE II

DIELECTRIC PROPERTIES OF BALANCED SALT SOLUTION

Temperature (°C)	Dielectric Constant	Conductivity (mmho/cm)	Loss Tangent
24	73.8	30.24	.301
26	72.3	29.85	.303
28	68.9	29.02	.309
30	65.9	28.30	.315
32	62.8	27.26	.319
34	58.5	25.89	.325
36	53.9	24.02	.327
38	49.1	22.23	.332
40	45.2	20.77	.337

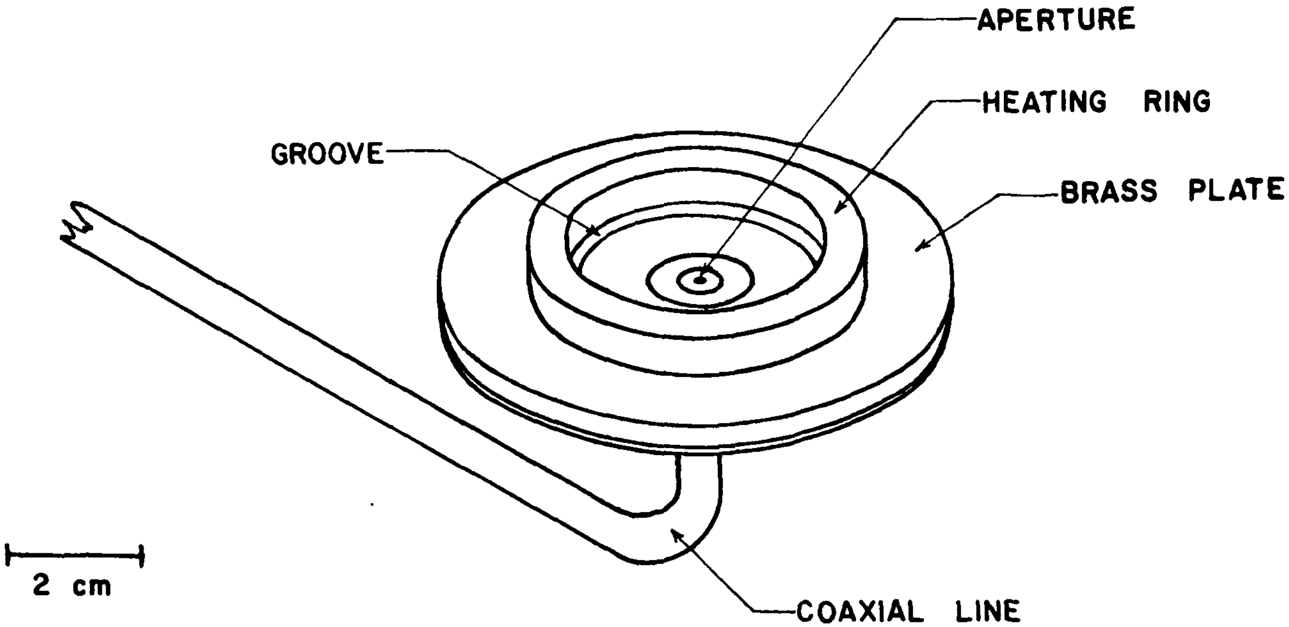


Figure 9. Isometric projection of coaxial exposure device showing 0.25-inch semi-rigid coaxial line, brass mounting plate, heating ring, and coaxial aperture. The outer edge of the culture dish rests in the small groove.

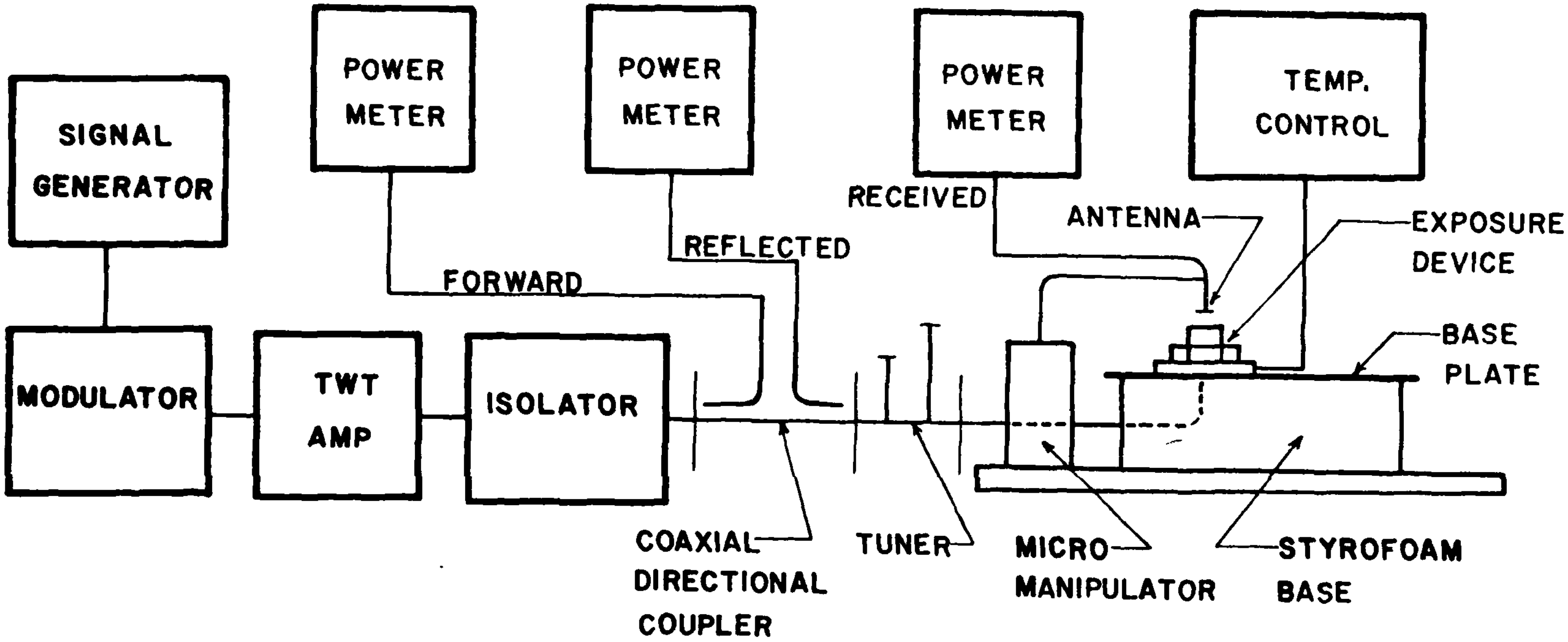


Figure 10. Laboratory setup used for characterization of the RFR coaxial exposure device. Configuration used for field measurements is illustrated. For temperature measurements, the received power meter and small probe antenna were replaced by temperature measurement instrumentation and a small high-resistivity thermistor probe, respectively.

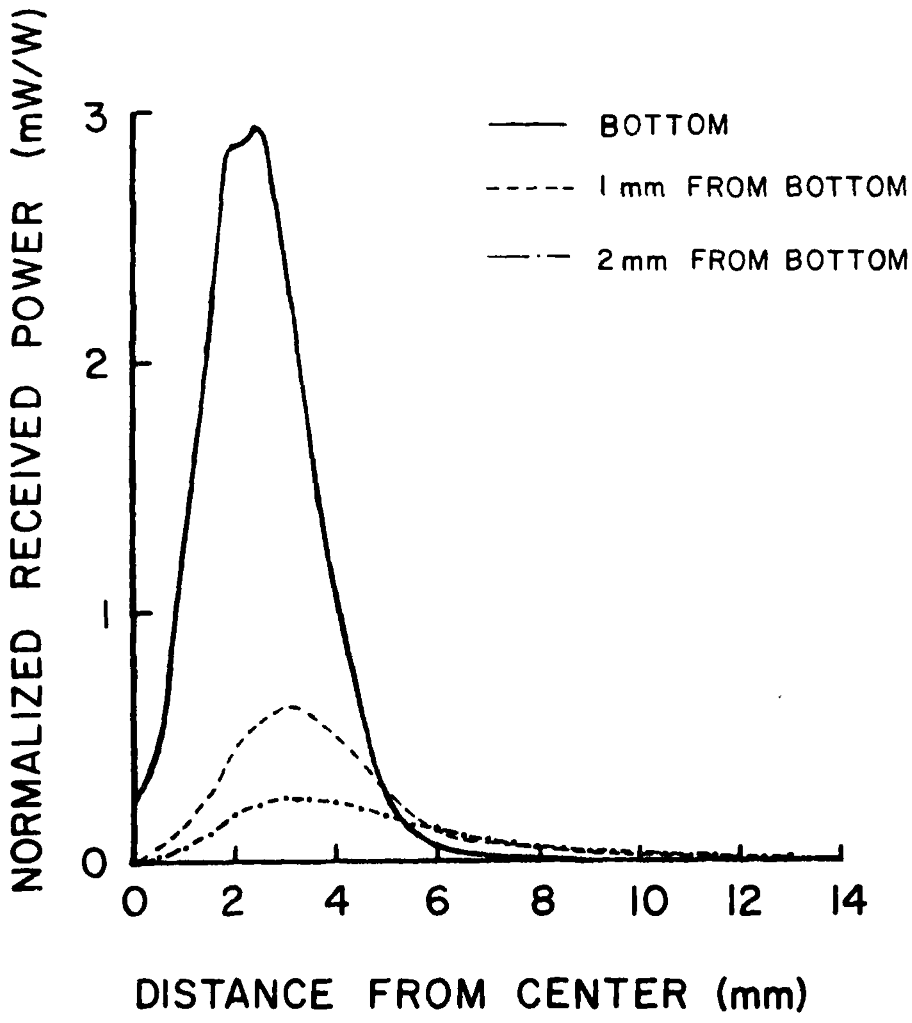


Figure 11. Normalized measured power in culture dish containing an aggregate medium (balance salt solution). Measurements were performed using a 4.3-mm long dipole probe.

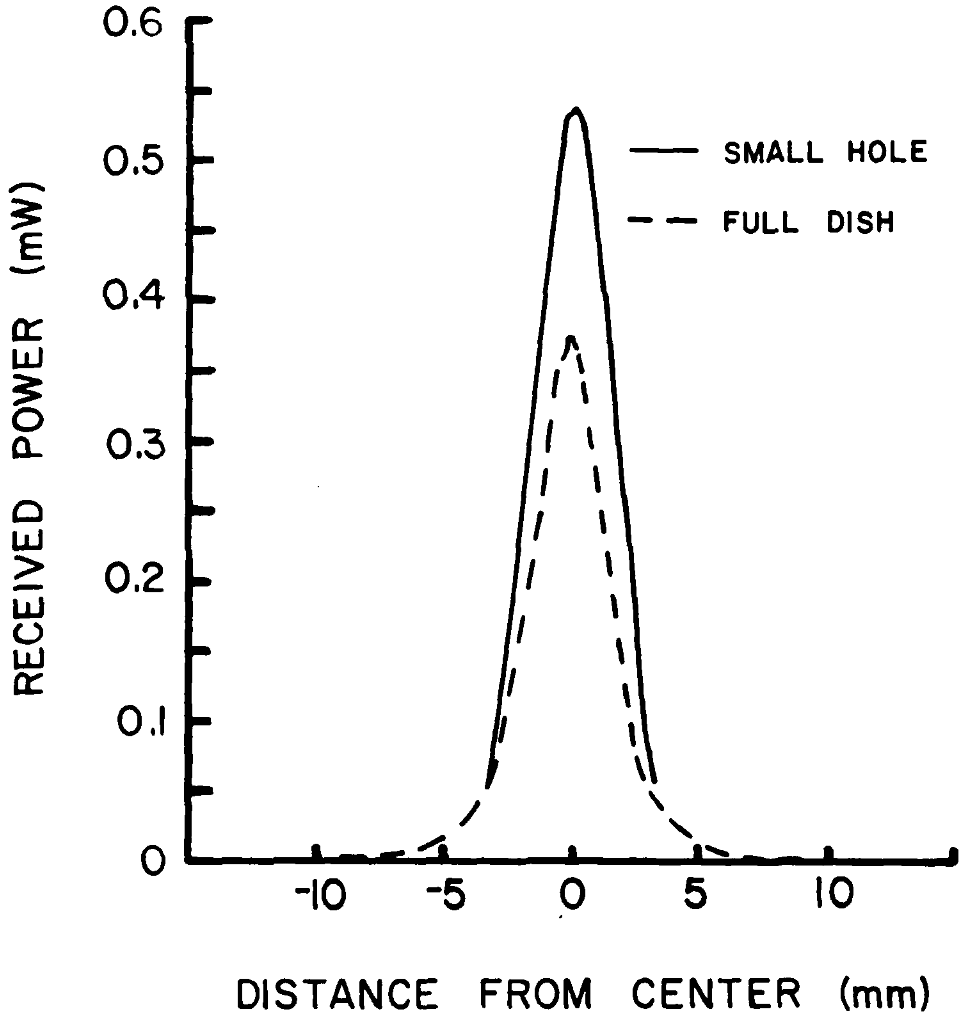


Figure 12. Normalized measured power in two culture dishes containing an aggregate medium (balanced salt solution [BSS]). Measurements were performed using a 2.7-mm long monopole probe positioned such that the center of the probe was 2 mm from the bottom of each dish.

Measurements of the temperature rise resulting from a rapid onset of microwave power were also performed using very small thermistors with nonmetallic leads to minimize field interactions [34-36]. An SAR was computed for each of several positions in the dish, with the maximum occurring at the center of the culture dish. Figure 13 shows the pattern of SAR derived from temperature measurements made at the bottom of the culture dish. The largest concentration of microwave energy (SAR = 2400 mW/g/W of input power) is at the bottom of the dish where the aggregates are placed. Reasonably good agreement was obtained between the powers received by the field probes and the computed SAR values.

The field intensity within the coaxial exposure device was also measured as a function of distance above the bottom of the culture dish for locations at the center of the dish and midway between the center and outer conductors of the coaxial device. The field intensity initially drops rapidly as a function of distance above the bottom of the dish. This is followed by a reduction in rate of decay such that the vertical field intensity is reasonably uniform at distances 2 mm off the bottom. The field intensities and SARs at points projected vertically above the bottom of the culture dish other than those measured were computed as a known fraction of the field intensities and SARs at the bottom of the dish.

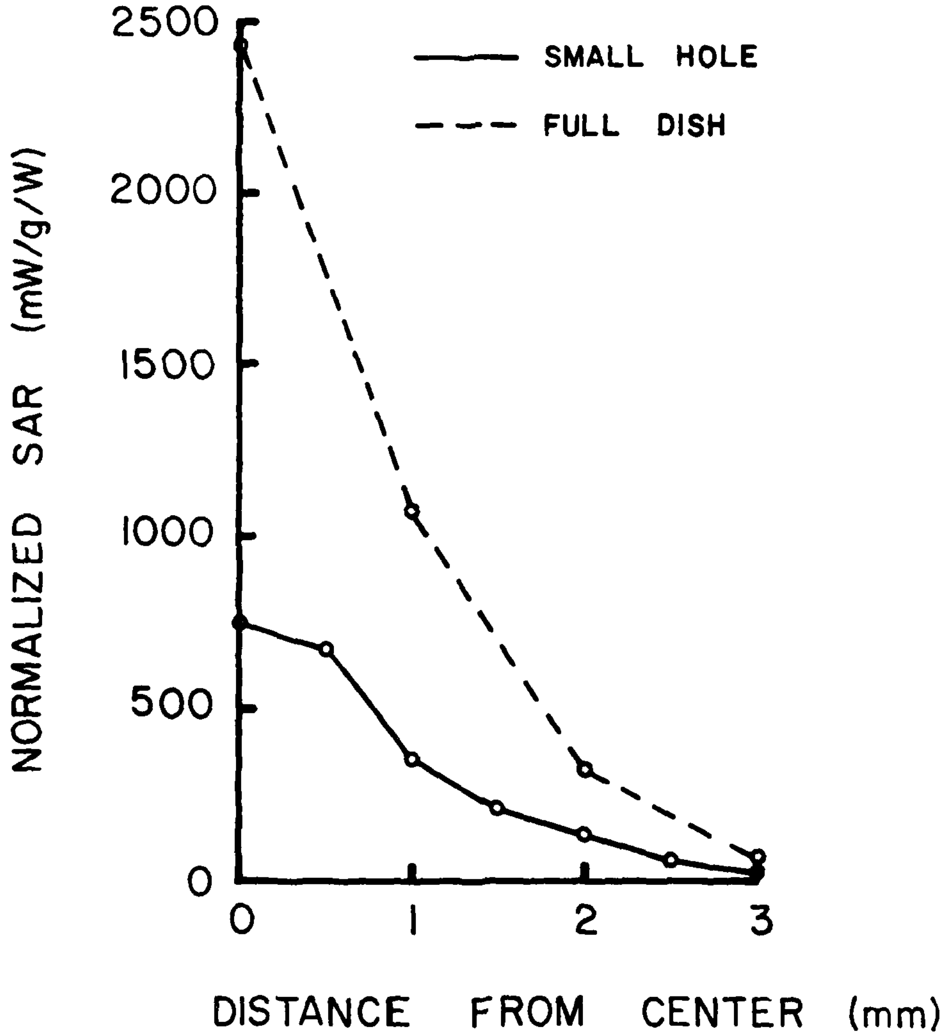


Figure 13. SAR at the bottom of two culture dishes normalized per watt of average input power to the coaxial exposure device.

SECTION IV

RADIOFREQUENCY RADIATION EXPOSURE RESULTS

Project efforts to date have been devoted to two major approaches. Our initial experiments were done with microelectrodes penetrating single aggregates and chains of aggregates which rested on the bottom of a culture dish. Later experiments utilized a photo-cell circuit to provide a voltage signal indicative of beat rate (or IBI) obtained from a television image of aggregates located on a platform above the dish's bottom. The RFR frequency was 2450 MHz in all experiments. In this section, all of these experiments are described and the results are presented.

In our first preliminary experiment, data on action potential characteristics were obtained from a single aggregate and two chains of aggregates on the dish bottom. One chain, consisting of five aggregates, was oriented in a radial direction and parallel to the major electric field component. The other chain, consisting of six aggregates, was oriented tangentially to an imaginary circle concentric with the coax, or orthogonal to the major electric field component. Action potentials were photographed from oscilloscope displays of the magnetic tape record of the experiment. The tape was replayed twice for each tape location with the oscilloscope sweep set at a fast rate for one replay and at a slower rate for the other. The fast sweep was used to image the upstroke of the action potential from which a maximum upstroke velocity was measured graphically. The slow sweep was used to image at least two action potentials from which maximum diastolic potential and maximum positive potential were measured graphically. The distance between action potentials on the slow sweep was used to compute beat rate (or conversely IBI).

Exposures to RFR in this experiment were 21 to 123 seconds in duration and were either CW or a gated CW. The gated CW consisted of a 60-ms pulse of CW power occurring 1.5 times per second, this rate being chosen to approximate expected firing rates. There were no changes in the parameters analyzed for three exposures of the chains to gated CW with SAR, averaged over time, of 64 to 1553 mW/g. Exposure to CW at SARs from 11 to 480 mW/g produced changes in parameters for all three aggregate configurations with a greater degree of change associated with larger SAR. The largest changes were in maximum upstroke velocity for all three configurations, maximum diastolic

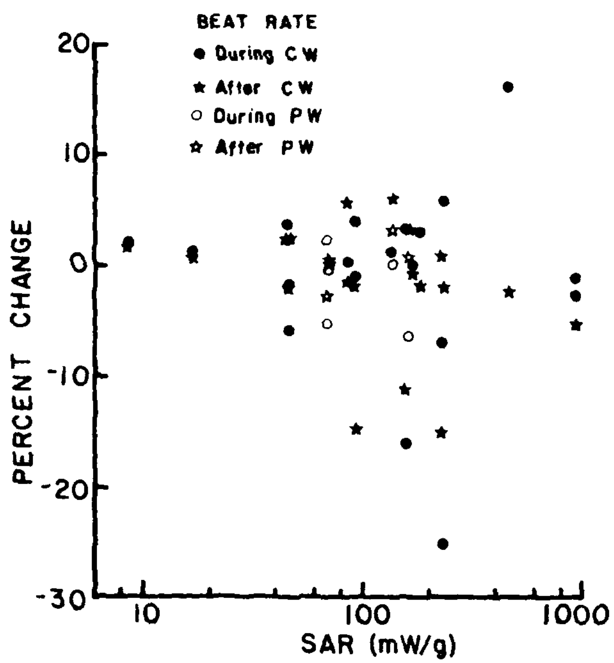
potential for the tangential chain, and beat rate for the single aggregate and radial chain. There were no changes in maximum positive potential for all configurations and none for beat rate in the tangential chain. Measured changes were considered to be indicative of possible effects and worthy of further investigation. Since changes were seen for all three aggregate configurations, it was decided to use single aggregates to pursue the basis for effects.

In the next experiment, in which a sustained successful electrode impalement was obtained, a single aggregate was exposed to 227 mW/g of CW radiation for 5.3 minutes before the experiment was terminated because of technical difficulties. Measurements of action potential parameters and beat rate were made as before. No significant changes were seen in any parameter.

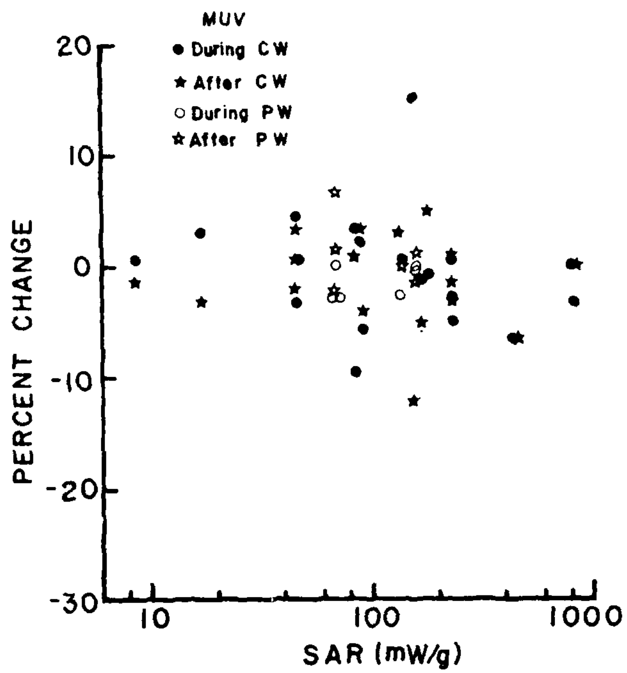
In another experiment, a single aggregate was exposed to 8.4 to 167 mW/g of CW radiation and 1.7 to 112 mW/g of pulsed radiation consisting of 10.9-μs pulses at a 10-KHz repetition rate. Exposure durations ranged from 30 sec to 90 sec for each modulation and the series of CW exposures was completed before the series of pulsed exposures. Progressively greater doses were applied in each series. Only very small changes were seen in the three parameters analyzed. Maximum positive potential was not analyzed in this experiment since it had seemed to be the least sensitive action potential parameter.

At this point, data on maximum upstroke velocity were combined and data on beat rate were combined. Data representing two cases were utilized. One case was the parameter value near the end of exposure and the other case was the parameter value a few seconds after exposure. Data for each parameter were expressed as percent changes from pre-exposure values. Figure 14 shows the results of this analysis, with data for CW and pulsed conditions separated and plotted against SAR. The most striking feature of these plots is the scatter in the normalized data. In both plots the degree of scatter seems to increase with SAR.

These same data are plotted in Figure 15 as means $\pm$ standard deviations for each SAR. Average change in beat rate is zero for small SAR but is generally a decrease at higher SAR's, which is opposite the anticipated effect due solely to positive bulk thermal changes at temperatures below 40°C (Figure 6). Variation in measured rate changes generally increases with SAR, as indicated by larger standard deviations. Average change in maximum upstroke

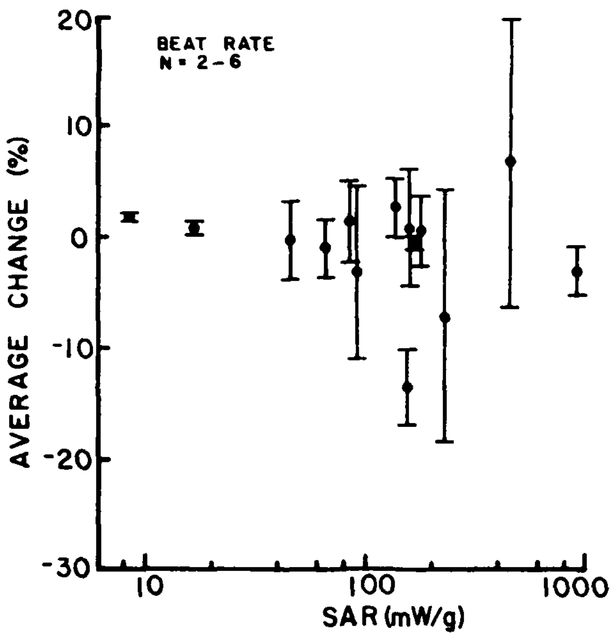


(a) Percent change in beat rate.

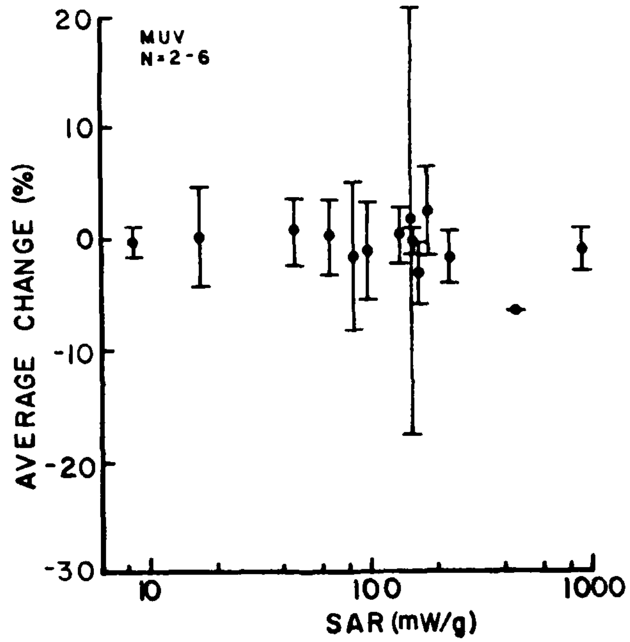


(b) Percent change in MUV.

Figure 14. Changes in (a) beat rate and (b) action potential maximum upstroke velocity (MUV) expressed as percent changes from pre-exposure values. Data are presented for exposures to continuous (CW) and pulsed (10.9 μs duration at 10 kHz) radiation and for values near the end of exposure (DURING) and immediately after exposure (AFTER). Data from three separate experiments.



(a) Average change in beat rate.



(b) Average change in MUV.

Figure 15. Changes in (a) beat rate and (b) action potential maximum upstroke velocity (MUV) of Figure 14 expressed as average changes in percent. Average $\pm$ standard deviation bars are shown. Data are grouped according to SAR irrespective of RFR modulation and time at which parameters were measured. Data from three separate experiments.

velocity is close to zero over all SAR, but the variation in measured changes is greatest for SAR between roughly 100 and 200 mW/g. These observations were considered tentative because of the limited amount of data and the variety of exposure conditions from which the data were taken. Trends in these data with SAR were nevertheless suggestive of statistical parameters which could be used to assess effects of exposure, especially in view of existing knowledge concerning variations in beat rate.

Our next experiment was designed to investigate the trends suggested by Figures 14 and 15. Exposure levels of 2 and 200 mW/g CW were used with a single aggregate at the dish bottom. Multiple exposures of 30 sec at each level were interspersed with 30 sec "sham exposure" periods during which no RFR was applied. Exposures and shams were separated by 30 sec. Data near the end of and immediately after an exposure or a sham were normalized to values measured just before the respective period. Based on the previously obtained pooled data, a change in maximum upstroke velocity or beat rate was not expected for the lower SAR. On the other hand, a reduction in average beat rate and increases in the variability of maximum upstroke velocity and of beat rate were expected at the higher SAR.

Figure 16 shows the results of this experiment. For each parameter, values of percent change during and after exposure or sham are presented as the average $\pm$ standard deviation. Average changes for shams were less than 1 percent. Average changes for 200-mW/g exposures were also within this range. Average maximum upstroke velocity decreases both during and after 2-mW/g exposure by about 2 percent. Average beat rate increased by about 1.5 percent during exposure and by about 1 percent just after exposure to 2 mW/g. These changes are undoubtedly statistically not significant because of the overlap of data within one standard deviation. Relative to values for shams, variability itself was slightly larger for maximum upstroke velocity under both exposure conditions. Variability in beat rate was smaller for 2 mW/g than for 200 mW/g with the values for the larger SAR similar to sham values. The larger variability for both beat

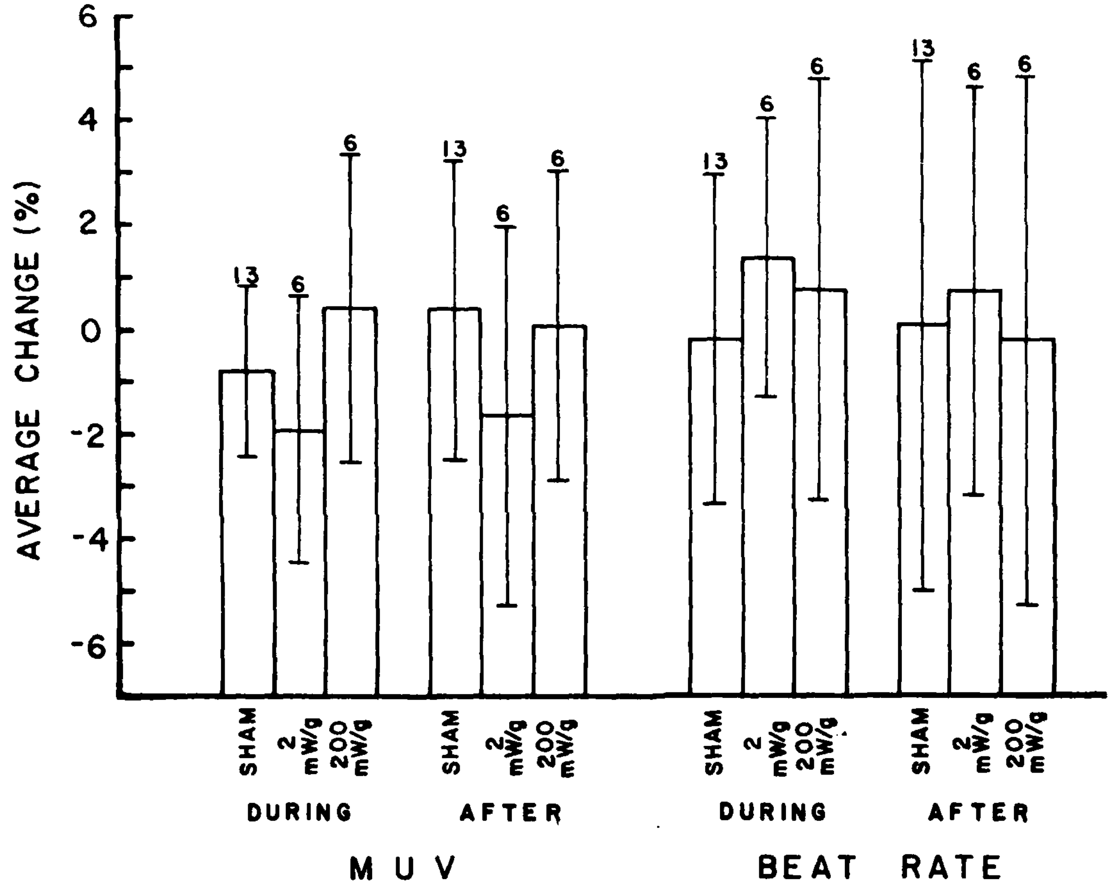


Figure 16. Average changes in beat rate and action potential maximum upstroke velocity (MUV) from one experiment. Average is represented by height of a column; bars indicate $\pm$ standard deviation. Data are shown for values near the end of exposure (DURING) and immediately after exposure (AFTER). Exposure was to 2 or 200 mW/g of CW radiation or to a sham period for the number of times indicated above a respective column.

rate and maximum upstroke velocity is consistent with the pooled data. However, interpreteration should be made cautiously because of the small magnitude of the changes and the different exposure periods used.

Data on the temperature dependence of maximum upstroke velocity and beat rate of the aggregate studied in this experiment were also obtained and are shown in Figure 17. Temperature was varied from about 37°C to 38.5°C. There is an increase in beat rate and a decrease in maximum upstroke velocity with temperature. The increase in beat rate is that expected from the curve in Figure 6.

From the evidence collected to this point, it became clear that, if an RFR effect was present, it was a subtle one, i.e., RFR produced no large, easily discernible change. Detection of a subtle change would be enhanced with a reduction of background variability in our experimental system and an increased sampling of parameter values. To reduce background variability, our next experiments involved the optical imaging of an aggregate and recording a signal proportional to aggregate contraction, using the system described in Section II. Although electrical activity was not observed, this method allowed the beat rate of an unperturbed aggregate to be monitored. Aggregates were placed on a small glass platform fashioned from a thin microscope cover slip resting on 1 x 1 x 5 mm glass supports. The top of the platform was 1.16 mm above the dish bottom. Effective SAR was reduced at this location for a given input power to the exposure device, but SAR capability was still sufficient to produce changes in aggregate parameters. One advantage of this platform was that aggregate SAR (at the height of the platform above the dish bottom) was more uniform than for aggregates placed on the dish bottom. The SAR was determined by scaling it from known values on the bottom using the ratio of powers received by a small horizontal dipole antenna and from "spot" measurements made at the level of the platform.

To increase the sampling of beat rate, computer programs were used to average 200 interbeat intervals as replayed from the recorded signal proportional to an aggregate's motion. Intervals were analyzed with a resolution of 5 ms. Programs were set up on a DEC PDP-11 computer with a DEC GT40 graphics terminal available in the Anatomy Department of Emory

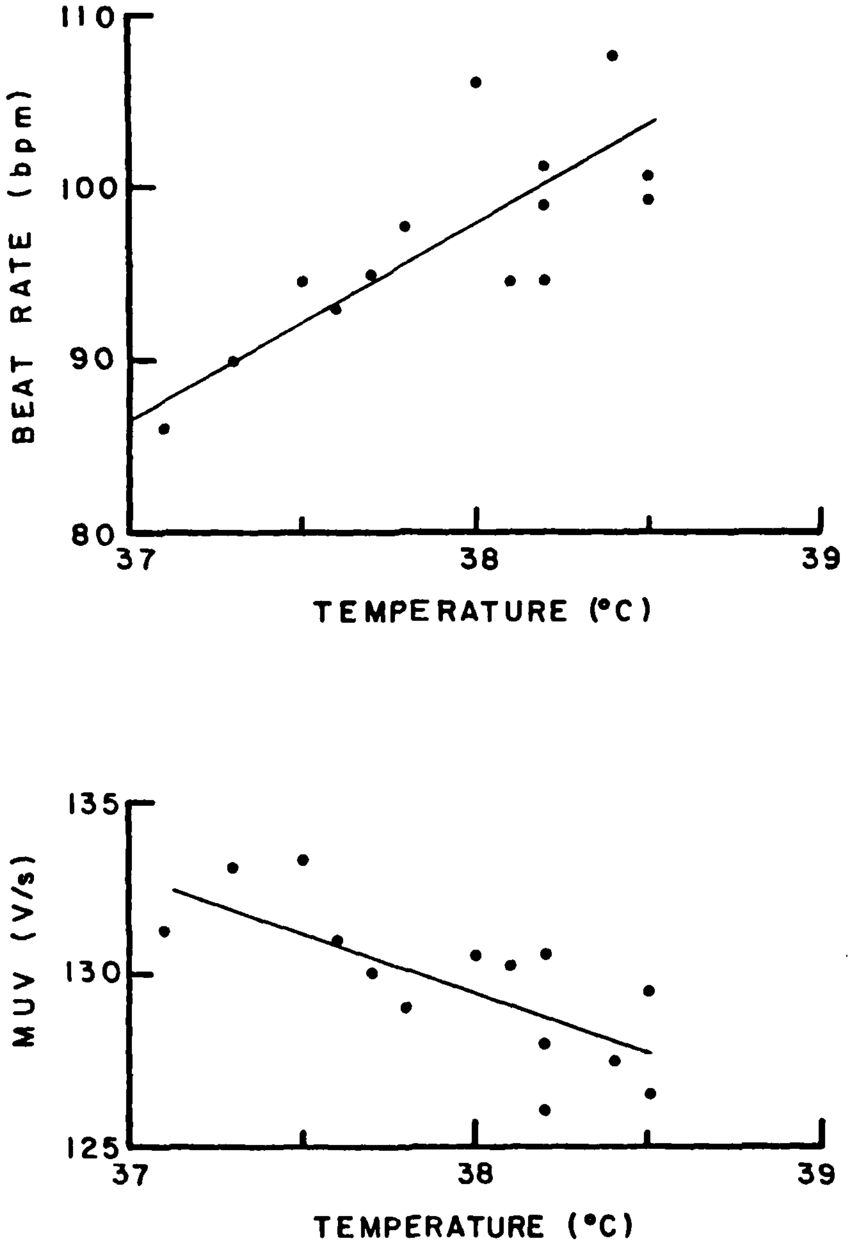


Figure 17. Beat rate (in beats per min) and action potential maximum upstroke velocity (volts per sec) studied as functions of temperature in the same experiment which produced the data shown in Figure 16.

University. Additional programming was necessary to adapt existing computer capabilities to our analysis. Various statistics in addition to average intervals were computed.

Figures 18 through 20 present statistics computed for interbeat intervals from one experiment in which several different exposure levels of CW RFR were applied to a chain of three aggregates. The first of these figures shows the mean $\pm$ standard deviation; the latter two, the coefficient of variance (CV). The value of CV is equal to standard deviation divided by the mean and is expressed in percent. For each exposure level, five separate periods were analyzed for each of two 5-min exposures. Individual periods encompassed the following times: (1) immediately before exposure, (2) at the beginning of exposure, (3) at the end of exposure, (4) immediately after exposure, and (5) starting approximately 3 min after exposure. Two hundred interbeat intervals (reciprocal of beat rate expressed per second) were analyzed for each point in the figures. The total period of time for a particular analysis depended on the average rate. Points are located at the approximate center of the period analyzed.

For the three smallest SARs - 0.3, 0.7, and 1.8 mW/g - there is no significant change in mean interval during exposure as shown in Figure 17. A small reduction is present at 4.4 mW/g. At the highest SAR used, 31.1 mW/g, the mean interval is clearly reduced during exposure and recovers after exposure. These decreases were expected from knowing the effect of increased temperature (see Figure 6). The coefficient of variance showed different changes during exposure (Figure 19) with the only possible pattern being an increase at the end of 4.4 mW/g and 31.1 mW/g exposure. To look further at a possible pattern in variance, the coefficients from the three lowest SARs were averaged together (Figure 20). It appears that there is no significant change in the coefficient of variance of SARs below those which produce bulk thermal changes in beat rate.

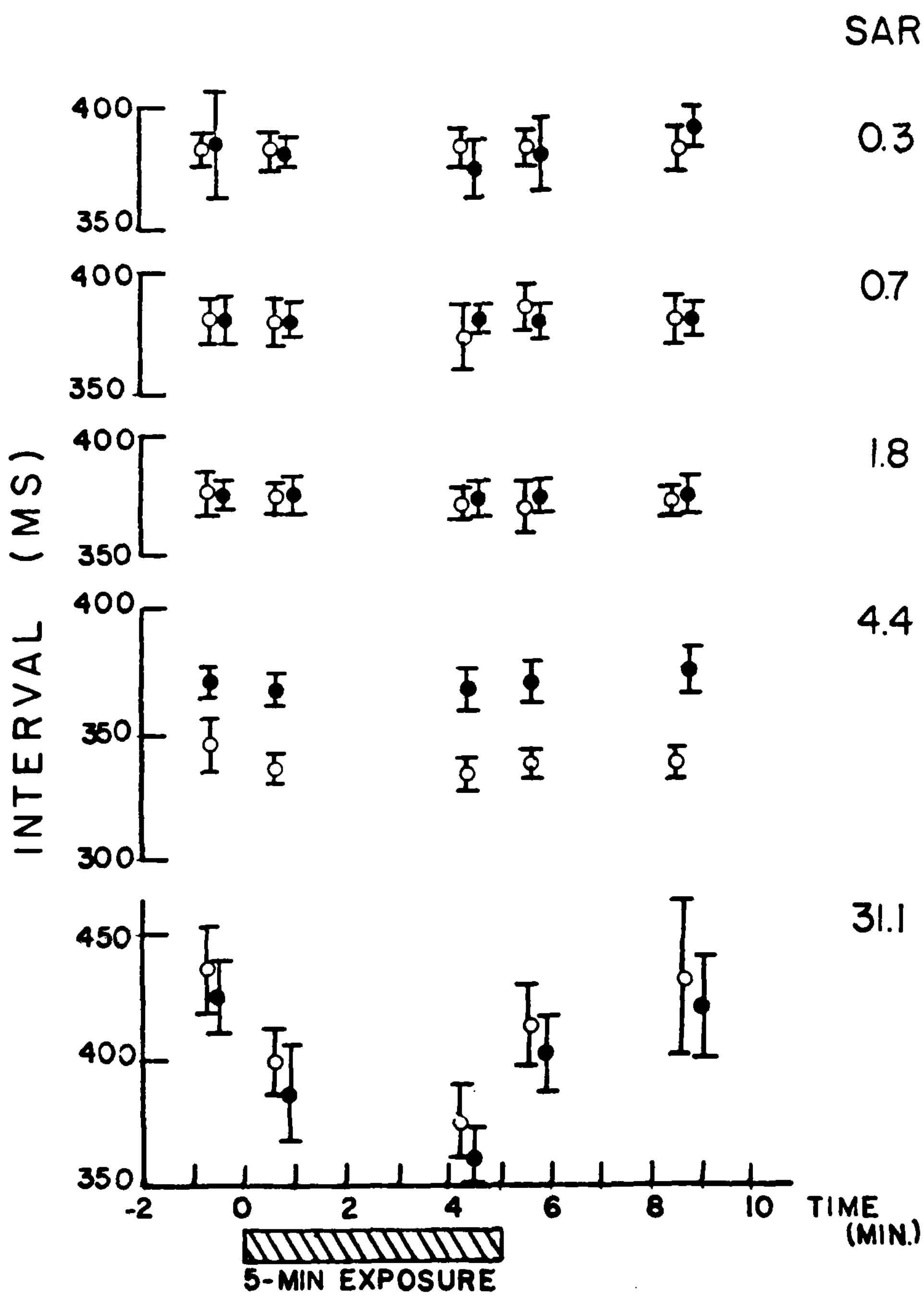


Figure 18. Interbeat intervals analyzed from the same experiment. Mean $\pm$ standard deviations are shown for different SARs. SAR is given in mW/g. Open circles represent data from the first of two exposures at a particular SAR; closed circles, from the second exposure.

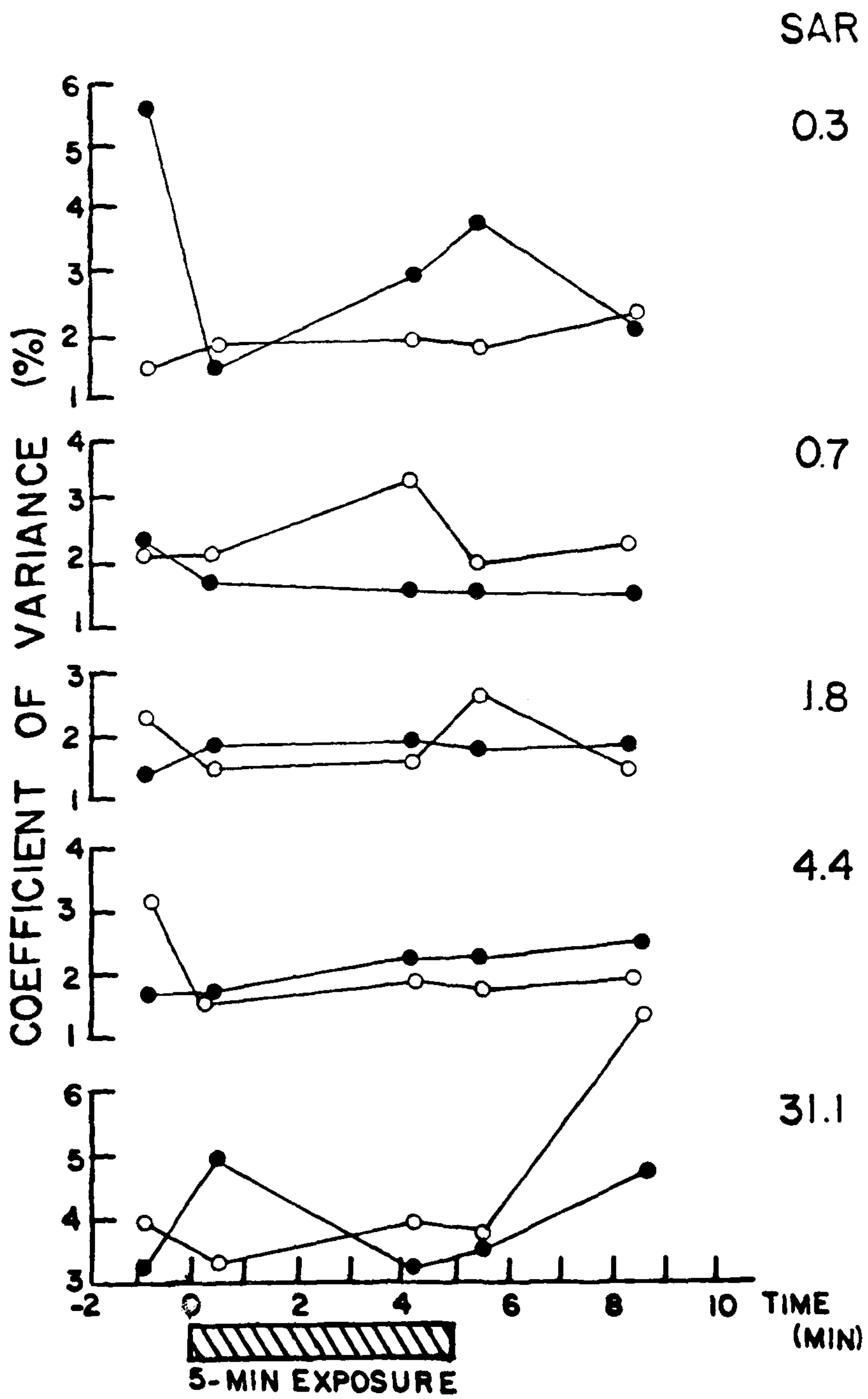


Figure 19. Coefficients of variance for the same data as in Figure 18. SAR is given in mW/g. Open circles represent data from the first of two exposures at a particular SAR; closed circles, from the second exposure.

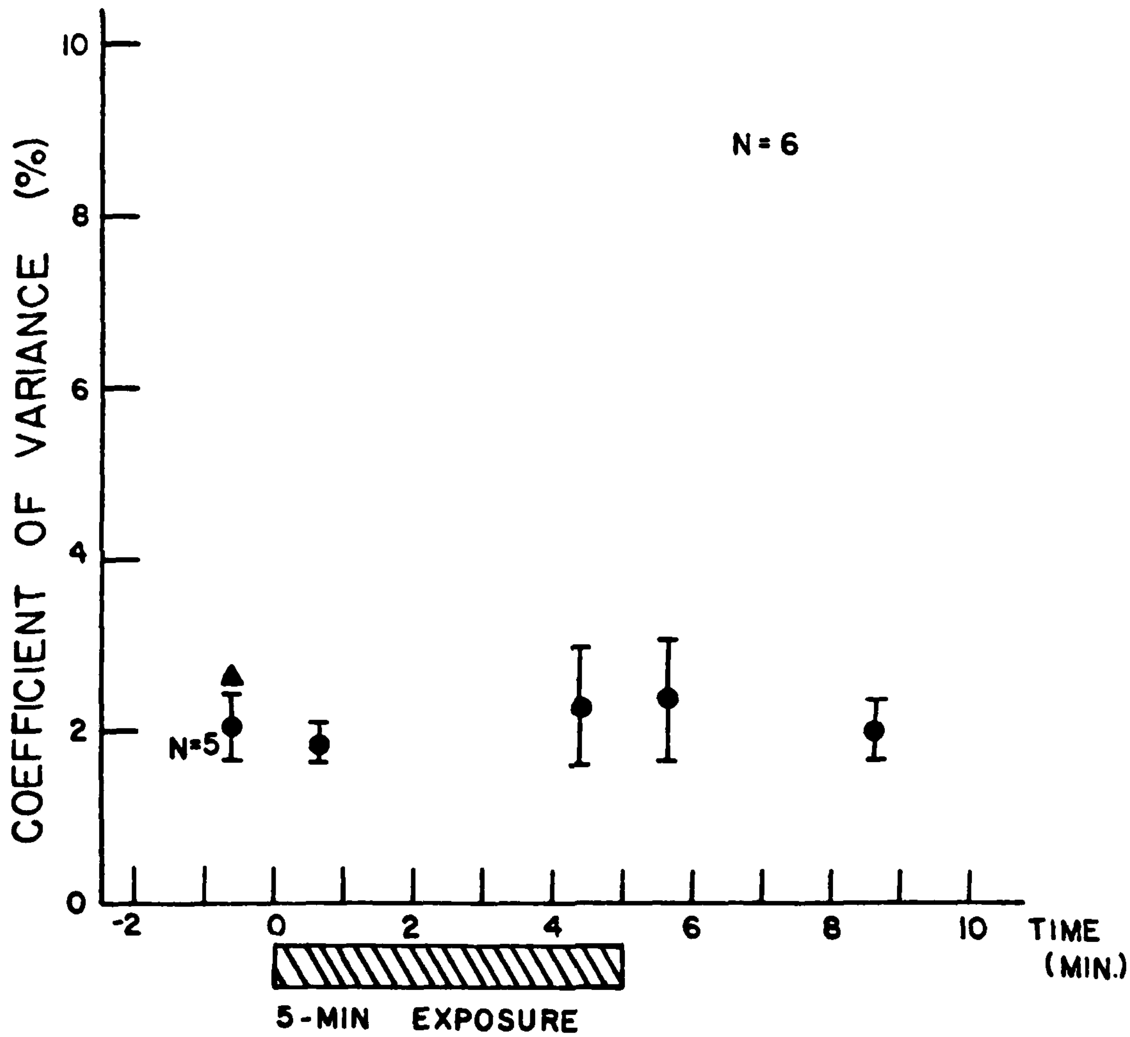


Figure 20. Means $\pm$ standard deviations for the coefficients of variance for the 3 smallest SARs in Figure 18. Data for the earliest period (pre-exposure) have been calculated with (triangle) and without (circle) the high value for 0.3 mW/g.

SECTION V

CONCLUSIONS AND RECOMMENDATIONS

The first year research efforts of the current research program to investigate the effects of pulsed and CW RFR on the electrical characteristics of cardiac-cell aggregates have been successfully completed. A well-characterized coaxial exposure system was developed for use in our investigation of potential RFR effects on cardiac-cell aggregates. The SAR and relative EM field intensity in the culture dish (which rests on the exposure device) were accurately determined as a function of position within the dish, for any given RF input power to the system. Thus, the dosimetry is accurately known for aggregates at any location in the culture dish. Similarly, a biological system for study was selected which has been extensively investigated for many years and whose biological and electrical properties are well known. The cardiac-cell aggregate system represents a model whose normal responses to external stimuli have been previously documented. In contrast to previous investigations which have employed biological preparations (such as dissected tissues or organs) which are dying while under study, the cardiac-cell aggregate model is a living, stable system which maintains its normal characteristics for periods of many hours, or even days. These combined features, development of a well-characterized system for cellular-level RFR exposure and a proven biological model system, make this approach using the cardiac-cell aggregate for investigations of RFR effects on excitable tissue electrical properties quite attractive.

The sensitivity of cardiac-cell aggregates' IBI to changes in bulk temperature was measured during the first year of this research program. It is concluded from these data that the aggregates offer a good system for the study of IBI effects due to small bulk thermal changes, and there are indications that the coefficient of variance of IBI may be increased

by small RFR-induced changes. No significant changes in average IBI or MUV were noted at a frequency of 2450 MHz for either CW or equivalent average power pulsed RF exposure levels below those which produced bulk temperature changes. However, at low levels of SAR, a statistical analysis of coefficient of variance suggests changes in IBI fluctuation that might be consistent with direct EM effects of RF energy on the random opening and closing of conductance channels. Also, it should be noted that in some experiments, low level RFR exposure produced small differences in MUV. It is noted that the coefficient of variance for IBI in several cases was increased by RFR. Further, the majority of the exposures were for CW radiation because of the need for establishing a baseline response to RFR of various average powers before investigating the specific response(s) of the aggregates to pulsed RFR.

Results and conclusions of investigations performed during the first year of this research program establish the attractiveness of the chosen biological model and the developed RF exposure system for studies of RFR-induced effects on excitable cells and tissues at the mechanistic level. Further, the baseline response of the biological model to RFR was established and a threshold for thermal responses was determined. This threshold for interbeat interval, which was studied in greatest detail, appeared to be approximately 4 mW/g SAR for changes in average interbeat intervals in our experiments. Further experiments will be done to determine effective SAR for other aggregate parameters which have not been studied in as much detail. Finally, methods for analysis of cellular electrical properties were established which are both statistically reliable and sensitive to RFR-induced effects. These results and conclusions also point to additional improvements in experimental design and to additional research which is necessary to better quantitate any RFR-induced effects on excitable cells which may exist and to identify the mechanism(s) underlying any observed interaction. To achieve the objectives of this research program and to better identify and understand the mechanism(s) for any observed effects, the following recommendations are made for the second year research efforts:

(1) Employ uniform exposure tissues times for both pulsed and CW radiation in each experiment.

(2) Repeat many of the CW RFR exposures performed during the first year efforts using pulsed radiation (both single aggregates and chains).

(3) Perform detailed computer statistical analyses for MUV in a manner similar to the analyses previously done for IBI.

(4) Perform studies of membrane voltage noise which provides a more sensitive measure of ion channel behavior than examination of IBI and MUV.

(5) Perform RFR exposures of single aggregates and aggregate chains at temperatures of 18° or 19°C to confirm that the previously measured fluctuations in IBI are due to RFR-induced bulk thermal effects.

(6) Perform studies to establish pharmacological agent/RFR interactions in cardiac-cell aggregates (both single aggregates and chains).

During the first-year research efforts, a number of different exposure durations were investigated in an effort to determine the sensitivity of any observed response of the aggregate electrical properties to this parameter. For future efforts, uniform exposure times(8-min duration) will be used in each experiment for both CW and pulsed exposure conditions. Much of the work involving RF exposures during the first year's investigations was performed to determine the baseline response of the aggregate electrical properties to RFR. It is recommended that many of the exposures performed using CW radiation be performed using pulsed RFR of the same average power. Additionally, it is recommended that both for the earliest exposures performed in the first year and for any future RFR exposures, detailed statistical analyses be performed for MUV and other action potential parameters similar to those performed for IBI. Because of the observed changes in the coefficient of variance under several exposure conditions, it is recommended that a membrane voltage noise analysis be performed for future

exposures. This analysis will provide a better indication of possible ion channel alterations due to RFR exposure than that obtainable from a fluctuation analysis of IBI.

Additional studies which are recommended include the exposure of cardiac-cell aggregates to RFR under conditions where the aggregate bathing medium temperature is in the range of 18°C. At this lower temperature, the IBI is much more sensitive to bulk temperature changes than at higher temperatures (see Figure 6). Experiments performed at these lower temperatures will enable us to confirm the nature (whether strictly bulk heating changes or whether RF-mediated changes occur) of the observed IBI fluctuations in experiments already performed. Also, studies of pharmacological agent/RFR effects are recommended. The modulating effects of RFR on the known pharmacological influences of agents such as tetrodotoxin may provide a more sensitive indication of RFR influences than to measure those influences directly.

SECTION VI

PROFESSIONAL STAFFING AND PUBLICATIONS

A. Project Professional Personnel

Everette C. Burdette, Principal Investigator
Biomedical Research Branch
Engineering Experiment Station
Georgia Institute of Technology

Ronald L. Seaman, Co-Investigator
Biomedical Research Branch
Engineering Experiment Station
Georgia Institute of Technology

Robert L. DeHaan, Co-Investigator and Program Director,
Emory University
Department of Anatomy
Emory University School of Medicine

R. Clark Lantz, Post-Doctoral Fellow
Department of Anatomy
Emory University School of Medicine

No advanced degrees were awarded during the 1 March 1979 - 29 February 1980 performance period.

B. Written Publications in Technical Journals

No papers were published in technical journals and no manuscripts were submitted for publication during the 1 March 1979 - 29 February 1980 performance period.

C. Interactions and Paper Presentations

The following interactions took place and paper presentations were made during the 1 March 1979 - 29 February 1980 performance period at the Review of Air Force Sponsored Basic Research In Environmental Protection Bioeffects held 15-17 January 1980, El Tropicano Motel, San Antonio, Texas (AFOSR Life Sciences Directorate).

1. "Investigation of Radiofrequency Radiation Effects on Excitable Tissues", E. C. Burdette, R. L. Seaman, and R. L. DeHaan (presented by E. C. Burdette).

2. "Alteration of Action Potential Parameters in Embryonic Heart Cell Aggregates by Radiofrequency Radiation", R. L. Seaman, E. C. Burdette, and R. L. DeHaan (presented by R. L. DeHaan).

The following abstracts based on the first year efforts were submitted in April 1980 for presentation at the Second Annual Meeting of the Bioelectromagnetics Society to be held 14-18 September 1980, San Antonio, Texas.

1. "Open-ended Coaxial Exposure System for Small Biological Preparations", E. C. Burdette, R. L. Seaman, and R. L. DeHaan.

2. "Radiofrequency Radiation Alteration of Cardiac-cell Aggregate Electrical Parameters", R. L. Seaman, E. C. Burdette, and R. L. DeHaan.

SECTION VII

REFERENCES

1. A. S. Presman, "Electromagnetic Fields and Life," Plenum Press, New York, 1970, pp. 336.

2. I. T. Kaplan, W. Metlay, M. M. Zaret, L. Birenbaum, and S. W. Rosenthal, "Absence of Heart-Rate Effects in Rabbits During Low-Level Microwave Irradiation," IEEE Trans. Microwave Theory Tech. MTT-19: 168-173, 1971.

3. L. Birenbaum, I. T. Kaplan, W. Metlay, S. W. Rosenthal, and M. M. Zaret, "Microwave and Infra-red Effects on Heart Rate, Respiration Rate and Subcutaneous Temperature of the Rabbit," J. Microwave Power 10:3-18, 1975.

4. S. Baranski and P. Czerski, "Biological Effects of Microwaves," Dowden, Hutchinson and Ross, Stroudsburg, PA, 1976, pp. 234.

5. A. H. Frey and E. Seifert, "Pulse Modulated UHF Energy Illumination of the Heart Associated with Change in Heart Rate," Life Sciences, Part II, 7:505-512, 1968.

6. R. M. Clapman and C. A. Cain, "Absence of Heart-Rate Effects in Isolated Frog Heart Irradiated with Pulse Modulated Microwave Energy," J. Microwave Power, 10 (4): 411-419, 1975.

7. L. M. Liu, F. J. Rosenbaum, and W. F. Pickard, "The Insensitivity of Frog Heart Rate to Pulse Modulated Microwave Energy," J. Microwave Power, 11(3):225-232, 1976.

8. J. L. Lords, C. H. Durney, A. M. Borg, and C. E. Tinney, "Rate Effects in Isolated Hearts Induced by Microwave Irradiation," IEEE Trans. Microwave Theory Tech., MTT-21:834-836, 1973.

9. C. E. Tinney, J. L. Lords and C. H. Durney, "Rate Effects in Isolated Turtle Hearts Induced by Microwave Radiation", IEEE Trans. Microwave Theory and Tech., MTT-24:1-18, January 1976.

10. R. G. Olsen, J. L. Lords and C. H. Durney, "Microwave-Induced Chronotropic Effects in the Isolated Rat Heart," Ann. Biomed. Eng., 5:395-409, 1977.

11. J. R. Reed, J. L. Lords, and C. H. Durney, "Microwave Irradiation of the Isolated Rat Heart After Treatment with ANS Blocking Agents," Radio Science, 12(6S):161-165, 1977.

12. H. Wachtel, R. Seaman, and W. Joines, "Effects of Low-Intensity Microwaves on Isolated Neurons," Ann. of the New York Acad. Sci., 247:46-61, 1975.

13. R. L. Seaman and H. Wachtel, "Slow and Rapid Responses to CW and Pulsed Microwave Radiation by Individual Aplysia Pacemakers," J. Microwave Power, 13(1):77-86, 1978.

14. R. J. MacGregor, "A Possible Mechanism for the Influence of Electromagnetic Radiation on Neuroelectric Potentials," IEEE Trans. Microwave Theory Tech.,MTT-27:914-921, 1979.

15. F. S. Barnes and C. L. Hu, "Model for Some Nonthermal Effects of Radio and Microwave Fields on Biological Membranes," IEEE Trans. Microwave Theory Tech.,MTT-25:742-746, 1977.

16. G. C. Berkowitz and F. S. Barnes, "The Effects of Nonlinear Membrane Capacity on the Interaction of Microwave and Radio Frequencies with Biological Materials," IEEE Trans. Microwave Theory Tech., MTT-27: 204-207, 1979.

17. W. F. Pickard and R. J. Rosenbaum, "Biological Effects of Microwaves at the Membrane Level: Two Possible Athermal Electrophysiological Mechanisms and a Proposed Experimental Test," Math. Biosci.,39: 235-253, 1978.

18. H. G. Sachs and R. L. DeHaan, "Embryonic Myocardial Cell Aggregates: Volume and Pulsation Rate," Dev. Bio.,30:233-240, 1973.

19. R. L. DeHaan and H. A. Fozzard, "Membrane Response to Current Pulses in Spheroidal Aggregates of Embryonic Heart Cells," General Physiol., Vol. 65, pp. 207-222, 1975.

20. R. D. Nathan and R. L. DeHaan, "Voltage Clamp Analysis of Embryonic Heart Cell Aggregates, " J. Gen. Physiol.,73:175-198, 1979.

21. D. L. Ypey, D. E. Clapham, and R. L. DeHaan, "Development of Electrical Coupling and Action Potential Synchrony Between Paired Aggregates of Embryonic Heart Cells," Membrane Biol.,51:75-96, 1979.

22. D. E. Clapham, A. Shrier, and R. L. DeHaan, "Junctional Resistance and Action Potential Delay Between Embryonic Heart Cell Aggregates," J. Gen. Physiol., in press, 1980.

23. R. D. Nathan, J. P. Pooler, and R. L. DeHaan, "Ultraviolet-Induced Alternations of Beat Rate and Electrical Properties of Embryonic Chick Heart Cell Aggregates,"J. Gen. Physiol., Vol. 67, pp. 27-44, 1976.

24. J. R. Clay and R. L. DeHaan, "Fluctuations in Interbeat Interval in Rhythmic Heart-Cell Clusters," Biophys. J.,28:377-390, 1979.

25. R. L. DeHaan and H. G. Sachs, "Cell Coupling in Developing Systems: The Heart-Cell Paradigm," Chapter 5 in Current Topics in Developmental Biology, Vol. 7, Academic Press, New York, 1972, pp. 193-228.

26. R. L. DeHaan and L. J. DeFelice, "Oscillatory Properties and Excitability of the Heart Cell Membrane," Theoretical Chemistry, Vol. 4, Academic Press, New York, 1978, pp. 181-233.

27. T. F. McDonald, H. G. Sachs, and R. L. DeHaan, "Development of Sensitivity to Tetrodotoxin in Beating Chick Embryo Hearts, Single Cells, and Aggregates," Science,176:1248-1250, 1972.

28. J. R. Clay, L. J. DeFelice, and R. L. DeHaan, "Current Noise Parameters Derived from Voltage Noise and Impedance in Embryonic Heart Cell Aggregates," Biophys. J.,28:169-184, 1979.

29. L. J. DeFelice and R. L. DeHaan, "Membrane Noise and Intercellular Communication," Proceedings of the IEEE, 65:796-799, 1977.

30. L. J. Elsas, F. B. Wheeler, D. J. Danner, and R. L. DeHaan, "Amino Acid Transport by Aggregates of Cultured Chicken Heart Cells. Effect of Insulin," J. Biol. Chem.,250:9381-9390, 1975.

31. R. D. Nathan and R. L. DeHaan, "In-Vitro Differentiation of a Fast Na^+ Conductance in Embryonic Heart Cell Aggregates," Proc. Natl. Acad. Sci., 75:2776-2780, 1978.

32. E. C. Burdette, F. L. Cain, and J. Seals, "In-Vivo Probe Measurement Technique for Determining Dielectric Properties at VHF Through Microwave Frequencies," IEEE Trans. Microwave Theory Tech., Vol. 28, No. 4, 1980.

33. E. C. Burdette, F. L. Cain, and J. Seals, "In-Vivo Determination of Energy Absorption in Biological Tissue," Final Technical Report, Project A-1755, U.S. Army Research Office, Grant No. DAAG29-75-G-0182, January, 1979.

34. R. R. Bowman, "A Probe for Measuring Temperature in Radio-Frequency-Heated Material," IEEE Transactions Microwave Theory and Techniques, 24:1, pp. 43-45.

35. J. C. Toler, E. C. Burdette, J. Seals, and F. L. Cain, "An Investigation of Thermistors for Temperature Mapping Biological Specimens During EM Radiation," Record of 1977 Symposium on the Biological Effects of EM Waves, Airlie, VA., October 30-November 4, 1977. p. 6.

36. E. C. Burdette, F. L. Cain, and J. J. Wang, "Electromagnetic Dosimetry: Development of Analysis and Measurement Techniques for Three-Dimensional Complex-Shaped Dielectric Bodies," Digest of 1979 URSI/Bioelectromagnetics Society Symposium, Seattle, Washington, June 1979, p. 374.